JOURNAL OF ANATOMY

UNIVERSITY OF MICHIGAN
NOV 9 1950
MEDICAL LIBRARY

ORIGINALLY THE JOURNAL OF ANATOMY AND PHYSIOLOGY

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND BY

J. C. BRASH
J. D. BOYD
W. E. LE GROS CLARK
F. GOLDBY
W. J. HAMILTON
T. NICOL
J. M. YOFFEY
C. M. WEST (EDITOR)

VOLUME 84
PART 4
OCTOBER 1950

Entered at New York Post Office as Second Class Matter

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE

Price Twelve Shillings and Sixpence net

Books ON
ANATOMY AND MEDICAL SCIENCES

of all publishers supplied from stock.
Catalogues on request. Please state interests.

LENDING LIBRARY
MEDICAL & SCIENTIFIC

NEW BOOKS AND LATEST EDITIONS ALWAYS OBTAINABLE

ANNUAL SUBSCRIPTION, TOWN OR COUNTRY, FROM ONE GUINEA

PROSPECTUS ON APPLICATION

H. K. LEWIS & Co. Ltd.
136 Gower St., LONDON, W.C. 1
Telephone: EUSton 4282 (5 lines)
Business hours: 9 a.m. to 5 p.m.
Saturdays 10 a.m.

MICHROME BRAND

STAINS & REAGENTS FOR MICROSCOPY

Azocarmine	Janus Blue
Azur I & II	Janus Green
Brilliant Cresyl Blue	Leishman Stain
Cedarwood Oil	May-Grunwald Stain
Field's Stain	Nile Blue Sulphate
Giemsa Stain	Rosolic Acid

Price List on application
Also Stains in tablet form
Slides, Coverslips, Dropping Bottles, Microtome Knives, Laboratory Sundries

Now ready:
Microscopic Staining Techniques
No. 1 (24 pp.) 1s. 6d. No. 2 (52 pp.) 3s. 6d.

EDWARD GURR, LTD.
108 WATERFORD ROAD
WALHAM GREEN, LONDON, S.W.6
Tel.: Renown 2011 Cables: Gurr, Renown 2011, London

UNIFORM HIGH QUALITY, RELIABILITY AND IMMEDIATE DELIVERY

TWO NEW ROYAL SOCIETY PUBLICATIONS

The Preparation of Scientific Papers
PREPARED FOR THE INFORMATION SERVICES COMMITTEE
2s. 6d. net

A List of British Scientific Publications Reporting Original Work or Critical Reviews
2s. 6d. net

*

Biochemical Aspects of Genetics
BIOCHEMICAL SOCIETY SYMPOSIA 4
The report of a meeting held in 1949. The contributors are J. B. S. Haldane, W. J. C. Lawrence, L. S. Penrose, C. Rimington, J. G. Carr, D. G. Catcheside, G. Pontecorvo, J. Monod, and A. Neuberger.
6s. net

CAMBRIDGE UNIVERSITY PRESS

A METHOD FOR THE STUDY OF ARTERIAL ANASTOMOSES

By D. A. McDONALD and J. M. POTTER
Department of Physiology, St Bartholomew's Hospital Medical College

Physiological aspects of anastomotic channels are not elucidated by ordinary injection methods. These merely reveal pathways which given streams of blood may or may not follow during life. We have studied in rabbits the complicated anastomosis of the circle of Willis, using a modification of the rapid coagulation technique devised by Franklin & Amoroso (1948).

This method was designed to demonstrate the distribution of blood throughout the body (or in a given organ) under any special conditions. A powerful blood coagulant mixed with a dye was introduced so that the 'active' part of the circulation would be clotted and could be seen after death. For instance, if the animal had previously received an adequate dose of adrenaline the post-mortem appearance of the dye, concentrated especially in the heart and lungs, and its relative lack in skin, muscles and viscera reflected the redistribution of blood which had occurred.

METHODS

The original method

Purified Russell's viper venom ('Stypven') was used in a solution of 1 mg./ml. distilled water solvent. This was injected intravenously, the dose being 1 mg./kg. body weight. With this had been mixed an equal volume of a solution of dye; 5% Evans blue (the isomer of Trypan blue, T 1824) was preferred because it remains in the vessels. An anaesthetized rabbit thus injected usually died within 3 min.

The modification for arterial injection

For the rapid arterial blood stream, purified thrombin (Upjohn) and crude Russell's viper venom were found to be better coagulants.

A rabbit, weighing about 2 kg., was anaesthetized with intravenous nembutal (30 mg./kg.) and inhaled ether. Thrombin 150 units, or crude venom 20 mg., in 1·0 ml. normal saline was mixed with an equal volume of 5 or 10% Evans blue. The injection was made slowly (0·5–0·75 ml./min.) by an indirect route (so that there was little interference with the normal blood flow) into either the internal carotid or vertebral system.

To inject the internal carotid artery, we inserted into the external carotid a fine cannula pointing towards the heart. The injected fluid then passed against the external carotid blood stream into the common carotid, and thence was carried up the internal carotid by the action of the heart. By keeping the column of dye in the common carotid constant the rate of injection could be matched against the velocity of the blood flow up the internal carotid.

The vertebral artery was similarly injected through a centrally pointing cannula in the axillary artery. To balance the pressures, the artery on the opposite side

which corresponded to that holding the cannula was also ligated, i.e. the external carotid or the axillary artery.

The carotid injections did not usually kill the animal, and death was then produced by clamping the ascending aorta. Unilateral vertebral injection, however, caused death rapidly (McDonald & Potter, 1948).

The head was severed and a small hole made in the skull to admit fixative. The cisterna magna also was opened and the head placed in 10% formol saline. The brain was carefully removed when fixed. The vessels in the neck were also carefully dissected to exclude any important anomaly.

RESULTS

Coagulation was not always satisfactory and never complete throughout the arteries injected, but two of our findings (McDonald & Potter, 1949*a*) give some indication of the value of the method. First, a clear demarcation was seen in the posterior communicating artery between dyed, coagulated blood from the injected system, and undyed, fluid blood. This demarcation was also seen on the surface of the hemisphere between the territories of the middle cerebral and posterior cerebral arteries. This is indirect evidence that it is here that carotid blood meets and opposes the vertebral blood stream.

Second, when a vertebral artery was injected, a distinct thread of dyed, coagulated blood was seen passing from the injected artery into the same side of the basilar artery. Dye and clot were seen in the branches of the basilar artery on that side alone. This was evidence that streamline flow of the arterial blood from the vertebral arteries occurs in the basilar artery. We have since confirmed this by direct observation in the living rabbit (McDonald & Potter, 1949*b*).

SUMMARY

The use of an intravascular coagulant with a dye for studying the behaviour of the blood flow in arterial anastomoses during life is described.

Two features of the arterial supply to the rabbit's brain are noted. One of these, the streamline flow of vertebral blood in the basilar artery, could not have been demonstrated by conventional injection methods.

We wish to thank Prof. K. J. Franklin, who suggested that we might make these studies. For the crude venom we are indebted to Drs J. W. Trevan, F.R.S., and A. C. White, of the Wellcome Foundation Research Laboratories, and for the thrombin (Upjohn) to Dr E. J. Cohn of Harvard University.

REFERENCES

Franklin, K. J. & Amoroso, E. C. (1948). Oral communication to the Physiological Society, 23 March.
McDonald, D. A. & Potter, J. M. (1948). Ménières Syndrome. *Brit. med. J.* **2**, 995.
McDonald, D. A. & Potter, J. M. (1949*a*). Blood flow in the circle of Willis. *J. Physiol.* **108**, 34*P*.
McDonald, D. A. & Potter, J. M. (1949*b*). Direct observation of streamlines in the basilar artery. *J. Physiol.* **109**, 17–18*P*.

SOURCES OF ARTERIAL BLOOD SUPPLY TO THE SUPERIOR AND MIDDLE CERVICAL SYMPATHETIC GANGLIA AND THE GANGLION INTERMÉDIAIRE

By E. L. PATTERSON
Anatomy Department, University of Manchester

INTRODUCTION

Standard anatomical works and even special monographs on the autonomic nervous system are singularly lacking in information about the blood vessels to the sympathetic. This is the more remarkable in view of the increasing clinical interest in this system and the surgical procedures associated with it. The text-books commonly mention only the ascending pharyngeal artery as the blood supply to the superior cervical sympathetic ganglion.

Adams (1942), in a historical review of the blood supply of nerves, has given a brief summary of previous work on the supply to the sympathetic ganglia.

During the course of the present investigation it became apparent that certain arteries, not, to the author's knowledge, previously noted in this connexion, may contribute extensively to the blood supply of certain parts of the sympathetic trunks. For example, in several cases examined such an unlikely vessel as the occipital artery provided a considerable arterial supply to the superior cervical ganglion. Again, on a number of occasions it was noticed that the lumbar sympathetic trunks derived a blood supply from unnamed retroperitoneal branches of the aorta and common iliac arteries in addition to the well-known segmental supply from the lumbar arteries. It was decided therefore to examine the frequency of the less common sources of supply and also to determine the more usual patterns of the arteries that are distributed to the individual ganglia. It was anticipated that since certain ganglia are notoriously variable in size and position there would be a concomitant variation in the arrangement of their blood vessels, and such is, of course, the case. On the other hand, when a ganglion of the sympathetic trunk is situated in a particular position it seems clear that its nutrient arteries commonly arise from certain regular sources and approach it in a certain manner.

METHOD

The arterial system in newborn infants has been injected with either indian ink or carmine-gelatin. The former, though excellent for the display of the intraneural vessels, has a tendency to pass through into the venous system. The most successful injections have been obtained by splitting the sternum, ligaturing the pulmonary trunk and inserting a cannula into the ascending aorta.

In estimating the frequency of any source of supply when using an injection technique, the principal source of error is the fact that occasionally, however much care is taken, small arterial branches fail to become filled. In order to minimize this error it is necessary to examine a large number of preparations and to report a source of supply as absent only when the parent artery and its branches appear to be well

injected and when no vessel (whether injected or not) can be traced from it to the sympathetic trunk by careful dissection. This is, of course, obvious and applies to any injection method, but it would appear that the number of preparations used by previous workers have, for this reason, not always been adequate. De Souza (1932), for example, based his observations on twelve foetuses.

The following account of the blood supply of the sympathetic ganglia in the neck is based on an examination of twenty-five preparations in which the success of the injection seemed beyond doubt. While this number is probably adequate to provide a picture of the common patterns of arterial supply, no reliable estimate can be given at this stage of the frequency of the less usual sources of ganglionic arteries.

OBSERVATIONS

The superior cervical ganglion

This ganglion derives its arterial supply most commonly from the ascending pharyngeal, superior thyroid and inferior thyroid arteries. Less usual sources are the occipital or the internal carotid arteries.

The ascending pharyngeal artery provided ganglionic branches in every case examined, and it usually takes the largest share in the arterial supply of this ganglion.

The superior thyroid artery also is a very common source, especially to the inferior part of the ganglion. Its contribution varies considerably but is often extensive. It contributed to the supply bilaterally in twenty-one of the twenty-five dissections.

The contribution from the inferior thyroid artery to the lower part of the ganglion was easily visible in eighteen of the twenty-five preparations. It was derived from either a branch of the ascending cervical artery, or from a muscular branch of the inferior thyroid given off on the medial side of the sympathetic trunk; after supplying branches to the middle cervical ganglion or the adjacent part of the trunk, it ascended on the interganglionic segment to the superior cervical ganglion. Sometimes both the above branches supplied the superior ganglion.

A free anastomosis between the various ganglionic arteries is often visible on both surfaces of the ganglion.

The ascending pharyngeal artery

This contributes branches to the ganglion in the following manner:

(1) One or more short medial branches arising directly from the parent vessel as it ascends on the medial side of the ganglion, pass laterally and, after primary divisions near the medial border of the ganglion, ramify on its anterior and posterior surfaces (Fig. 1). One or more of these branches may sink directly into the substance of the ganglion. The presence and number of these short medial branches depend on the level of origin of the ascending pharyngeal artery relative to the ganglion; if this origin is high, these branches are commonly absent (Fig. 2).

(2) A medial descending branch arises high up near the medial border of the ganglion, in most cases from the lowest prevertebral branch of the ascending pharyngeal artery before this passes posterior to the ganglion (Figs. 1–4). This medial descending branch passes down on either the anterior or posterior surface of the ganglion, along the medial border, distributing branches to both surfaces and

along one or more of the pharyngeal branches of the ganglion. When the origin of the ascending pharyngeal artery is high (Fig. 2) the medial descending branch is well marked and replaces the short direct medial branches from the parent artery.

(3) An anterior descending branch is often seen passing down on the anterior surface of the ganglion (Figs. 3–5), usually near the middle but sometimes nearer to its lateral border (Fig. 1). It is brought into view when the vagus and hypoglossal nerves are lifted away from the anterior surface of the ganglion, and it branches freely on this surface, anastomosing with the other ganglionic arteries near it. This

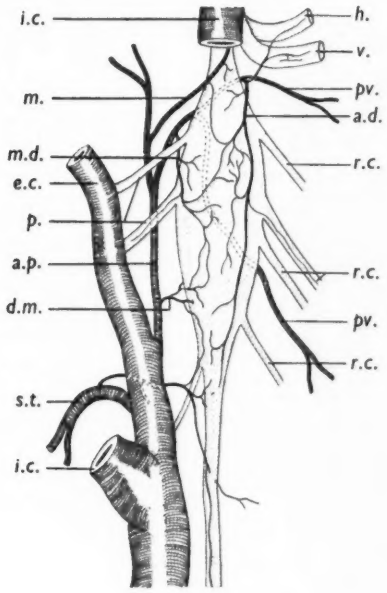


Fig. 1.

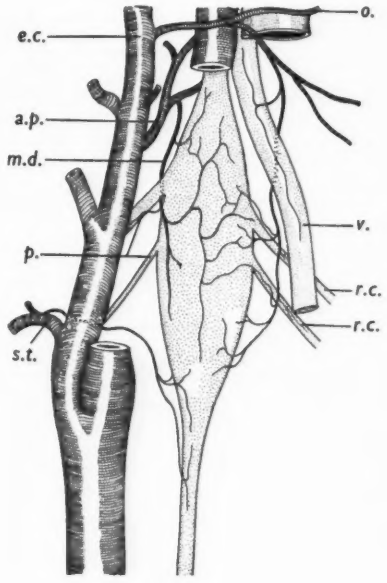


Fig. 2.

Fig. 1. Drawing from a dissection of the left superior cervical ganglion. *a.d.* anterior descending ganglionic artery; *a.p.* ascending pharyngeal artery; *d.m.* direct medial ganglionic artery; *e.c.* external carotid artery; *h.* hypoglossal nerve; *i.c.* internal carotid artery; *m.* meningeal branch of ascending pharyngeal artery; *m.d.* medial descending ganglionic artery; *p.* pharyngeal branch of ganglion; *pv.* prevertebral branch of ascending pharyngeal artery; *r.c.* ramus communicans; *s.t.* superior thyroid artery; *v.* vagus.

Fig. 2. Drawing from a dissection of the left superior cervical ganglion. *o.* occipital artery.

vessel in most cases arises from one of the higher prevertebral branches of the ascending pharyngeal artery near the superior end of the ganglion and reaches the anterior surface of the latter by turning round its lateral border. In one dissection, however, it arose at a much lower level from the trunk of the ascending pharyngeal and looped in a lateral direction in front of the internal carotid artery to reach the interval between the vagus and the ganglion (Fig. 5). In every case in which this anterior descending branch was present it contributed a substantial nutrient artery to the vagus and usually to the hypoglossal nerve also.

(4) Lateral branches arise in most cases from the lowest prevertebral branch of the ascending pharyngeal artery which, after passing laterally behind the ganglion, gives off a descending vessel. This curves downwards a short distance from the lateral border of the ganglion, being crossed superficially, less commonly deeply, by one or more of the laterally directed rami communicantes. This descending artery usually provides several vessels that pass medially on to the anterior surface of the ganglion and contribute extensively to the arterial net (Figs. 3–5). Lateral branches to the posterior surface of the ganglion are generally smaller and are less constant. It was noted that in several cases where the anterior descending branch was large and

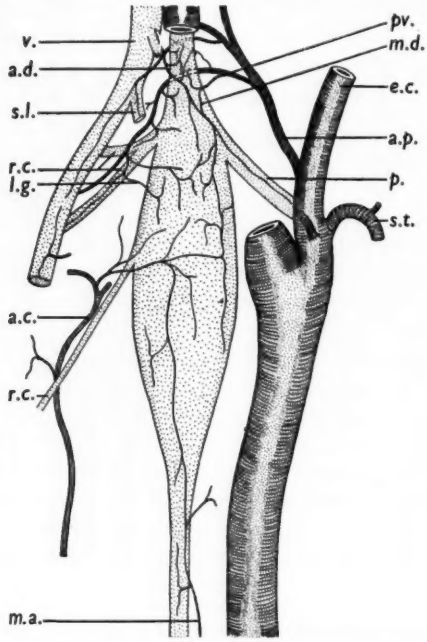


Fig. 3. Drawing from a dissection of the right superior cervical ganglion. *a.c.* ascending cervical artery; *l.g.* lateral ganglionic branch of ascending pharyngeal artery; *m.a.* medial ascending branch of inferior thyroid artery; *s.l.* superior laryngeal nerve.

situated near the lateral border of the ganglion, the lateral branches were scanty or absent altogether (Fig. 1).

(5) Posterior branches are in most cases small and less regular in position. They arise chiefly from the prevertebral branches of the ascending pharyngeal artery, as these pass laterally posterior to the ganglion and they ramify on the deep surface of the ganglion. The arterial net on this surface is thus less extensive than that on the anterior surface.

(6) Small branches are distributed to the extreme upper end of the ganglion, chiefly on its anterior surface. They arise from the anterior descending branch near its origin (Fig. 3) and also, in some cases, from a meningeal branch of the ascending

pharyngeal artery that passes across the superior pole of the ganglion (Fig. 1). Small vessels to the superior pole may also spring independently from a prevertebral branch of the artery that commonly takes a lateral course behind the upper part of the ganglion (Figs. 3, 4).

The ascending pharyngeal artery and its prevertebral branches form therefore an incomplete arterial ring within which a considerable part of the superior cervical ganglion is situated and from which the ganglion may receive nutrient vessels over the whole of its extent. In no case did this artery fail to contribute extensively to the vascularity of the ganglion.

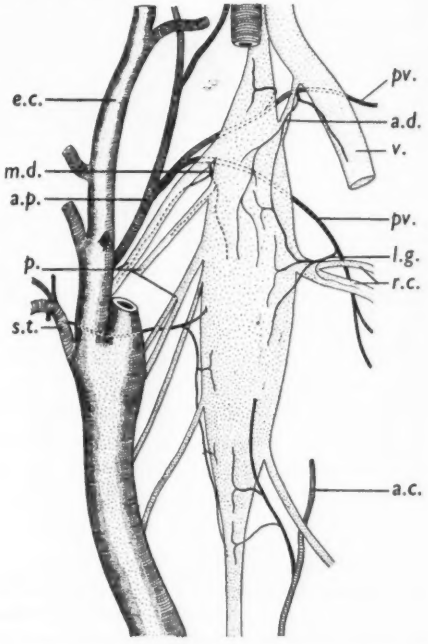


Fig. 4. Drawing from a dissection of the left superior cervical ganglion.

The superior thyroid artery

In the majority of cases (twenty-one out of twenty-five dissections) the superior thyroid artery contributes to the blood supply of the lower pole of the superior cervical ganglion, but the size of its contribution is very variable and in a few of the dissections it gave no ganglionic branch, its territory having been taken over by another vessel. The ganglionic branch commonly arises at or near the summit of the loop of the superior thyroid artery and takes a recurrent course in a postero-lateral direction, passing behind the two carotid arteries at the level of the carotid bifurcation or a short distance above it (Figs. 1, 2, 4 and 5). As it approaches the ganglion it commonly divides into superior and inferior branches. The former ramifies on the inferior part of the ganglion, participating freely in the arterial net, while the latter

turns downwards on to the trunk below the ganglion. This descending branch often becomes directly continuous with an ascending longitudinal vessel from the inferior thyroid artery. It is common to see a small artery arising from the sympathetic branch of the superior thyroid artery and accompanying the superior cervical sympathetic cardiac nerve distally.

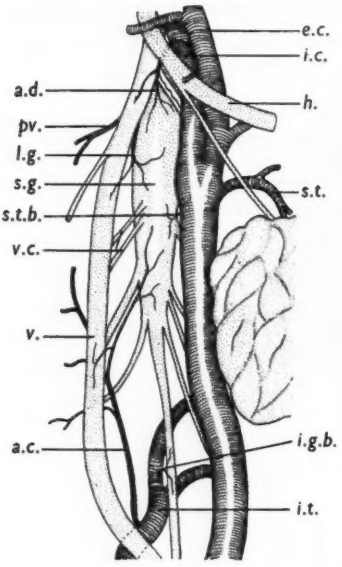


Fig. 5.

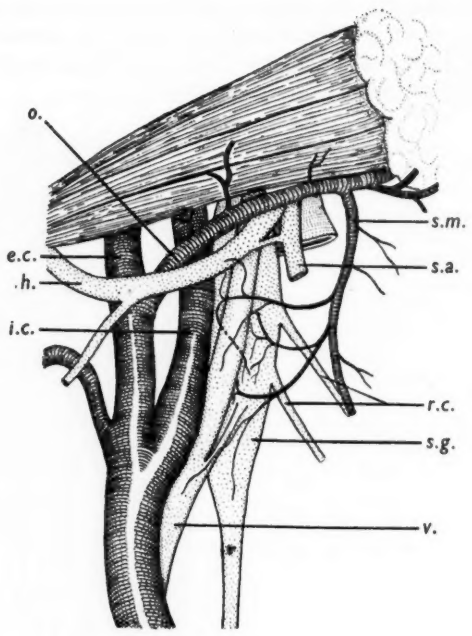


Fig. 6.

Fig. 5. Drawing from a superficial dissection of the right superior cervical ganglion. The only unusual feature is a branch of the ascending pharyngeal artery looping across the front of the internal carotid before dividing into a nutrient vagal artery, an arteria comes of the superior laryngeal nerve and the anterior descending ganglionic artery. *i.g.b.* interganglionic branch of inferior thyroid artery; *i.t.* inferior thyroid artery; *s.g.* superior cervical ganglion; *s.t.b.* recurrent ganglionic branch of superior thyroid artery; *v.c.* vagal communicating branch of ganglion.

Fig. 6. Drawing from a superficial dissection of the left superior cervical ganglion. *s.a.* spinal part of accessory nerve; *s.m.* sternomastoid branch of occipital artery.

The inferior thyroid artery

In approximately half the preparations examined (twelve out of twenty-four dissections) the ascending cervical artery contributed to the vascularity of the superior cervical ganglion (Figs. 3–5). In a few of these cases the ascending cervical artery was not actually a branch of the inferior thyroid artery but arose independently from the thyro-cervical trunk or from the transverse cervical artery. The branch concerned approaches the sympathetic trunk from the lateral side and reaches it at or a little inferior to the lower pole of the superior cervical ganglion, running in company with the rami communicantes in this region and branching to supply the

lower part of the ganglion. Occasionally the branch joins the sympathetic trunk some distance below the ganglion and ascends within its sheath.

In those cases in which the ascending cervical artery does not supply the superior ganglion, and occasionally when it does, a longitudinal vessel arises from the loop of the inferior thyroid artery on the medial side of the sympathetic trunk (Fig. 9) and ascends on the trunk to the superior ganglion. Here it anastomoses with a descending vessel from the arterial net (Fig. 3) or from the sympathetic branch of the superior thyroid artery. During its ascent this longitudinal artery often takes a spiral course, being related to the postero-medial surface of the sympathetic trunk at first and moving on to the anterior surface superiorly. Small branches arise from it at intervals, dividing in a T-shaped manner into ascending and descending twigs on the adjacent interganglionic segment of the trunk. As far as the superior cervical ganglion is concerned the vessel appears to have largely an anastomotic function, since it does not normally ramify on the ganglion before joining the descending vessel.

The occipital artery

No mention of this artery as a possible source of supply to the superior cervical ganglion has been found in reviewing reports of previous work, yet in three cases among the small series of twenty-five dissections examined this artery provided several branches to the ganglion. The branch of the occipital artery concerned is either the superior sternomastoid artery (Figs. 2, 6) or a descending muscular branch arising after the parent artery has crossed the internal jugular vein. In either case the vessel descends on the lateral side of the ganglion, distributing branches both to the sternomastoid and to the surrounding connective tissues and several branches that pass medially behind the internal jugular vein to ramify freely on the anterior surface of the ganglion, participating in the general arterial network. The vagus also receives a direct branch from this artery. Some of the ganglionic branches, as they approach the ganglion, cross the rami communicantes running infero-laterally from its lateral border, and small vessels from the branches may accompany these rami to reach the ganglion. They may also provide small vasa nervorum that accompany the rami towards the periphery (Fig. 2).

It was noted that in those cases in which the occipital artery took over the arterial supply of the ganglion from the lateral side, the prevertebral branch of the ascending pharyngeal artery that in most cases descends in a similar position to the lateral side of the ganglion was absent. It appears, therefore, that it is not very uncommon for the occipital artery to provide the lateral ganglionic branches that more usually arise from a prevertebral branch of the ascending pharyngeal artery.

The internal carotid artery

It appears to be quite unusual for the superior cervical ganglion to receive an arterial supply from the internal carotid artery, but this was seen in one specimen. In this case the ganglionic branch arose from the posterior aspect of the carotid sinus and very shortly divided into ascending and descending branches, each of which ramified on the anterior surface of the ganglion. It also provided a longitudinal artery to the vagus.

The middle cervical ganglion and ganglion intermédiaire

The middle cervical ganglion, being more subject to variation in position than the superior ganglion, naturally exhibits concomitant variations in the arrangement of its arterial supply. It was, however, apparent that there was some regularity in the arrangement of the ganglionic arteries for certain of the more common positions of the ganglion. In every instance these vessels arose exclusively from the branches of the thyro-cervical trunk, and especially from the inferior thyroid artery, with the exception of occasional cases in which the costo-cervical trunk contributed to a ganglion intermédiaire or to a middle cervical ganglion situated in a similar low position.

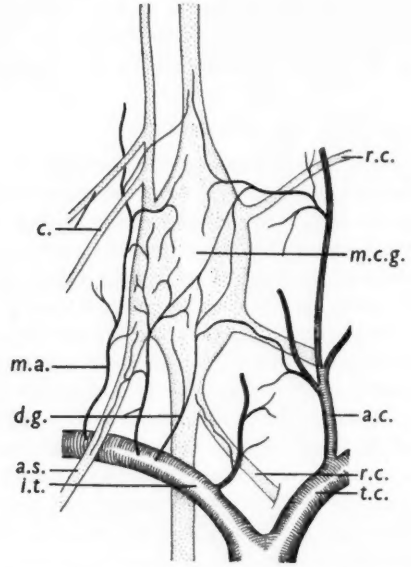


Fig. 7. Drawing from a dissection of the left middle cervical ganglion. *a.s.* ansa subclavia; *c.* cervical cardiac branch; *d.g.* direct ganglionic branches of inferior thyroid artery; *m.c.g.* middle cervical ganglion; *t.c.* transverse cervical artery.

When the middle cervical ganglion is situated immediately superior to the inferior thyroid artery, one may expect to find the arteries to it arranged as follows (Figs. 7–9):

(1) A branch of the inferior thyroid artery, arising on the medial side of the sympathetic trunk, ascends on the medial side of the ganglion and, in this situation, sends one or more branches laterally on to the ganglion, where they divide into ascending and descending vessels, participating in the superficial arterial net. Usually they ramify chiefly on the anterior surface of the ganglion, but one or more may be distributed to the posterior surface. The ascending artery from which the ganglionic vessels arise commonly continues its course to a higher level and is largely distributed to the adjacent prevertebral muscles. Very frequently, however, it provides an additional sympathetic branch that ascends on the sympathetic trunk

to the arterial net on the superior cervical ganglion, as previously mentioned. As this vessel ascends on the interganglionic segment small twigs arise from it, dividing dichotomously among the nerve fibres (Fig. 9).

(2) One or two branches arise directly from the inferior thyroid artery, usually from its anterior aspect, adjacent to the point at which the sympathetic trunk and inferior thyroid artery are in intimate relationship. These vessels ascend and ramify chiefly on the anterior surface of the ganglion, although occasionally they have quite a substantial distribution to its posterior surface. In the majority of the preparations in which this position of the ganglion was found, these direct branches appeared to contribute more extensively to the arterial network on the ganglion than the medial branches mentioned above.

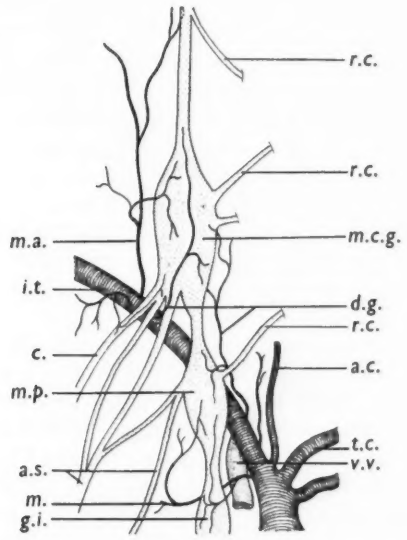


Fig. 8. Drawing from a dissection of a left middle cervical ganglion in three parts, the most inferior being the ganglion intermédiaire. *g.i.* inferior portion (ganglion intermédiaire) of middle cervical ganglion; *m.* medially directed muscular branch of inferior thyroid artery; *m.c.g.* superior portion of middle cervical ganglion; *m.p.* middle portion of middle cervical ganglion; *v.v.* vertebral vein.

(3) The ascending cervical artery quite commonly sends one or more slender branches to the ganglion from the lateral side, some of which may accompany the rami communicantes. This source of supply was found in nine out of nineteen dissections in which the ganglion occupied this situation. In a number of cases, however, the ascending cervical artery lies considerably lateral to the sympathetic trunk, and it is then unusual for it to supply the middle cervical ganglion, or indeed the sympathetic trunk at a higher level.

In those cases in which no ganglion is present immediately above the level of the inferior thyroid artery, the sympathetic trunk nevertheless normally receives in this situation a small artery that divides upon it dichotomously (Fig. 5). This vessel may

be a short branch arising directly from the inferior thyroid artery or from the medial ascending muscular branch of the latter to which reference has been made.

When the middle cervical ganglion, either in its entirety or as a ganglion intermédiaire, is situated below the inferior thyroid artery, it is more difficult to determine a regular pattern in the arrangement of its arteries. This is chiefly because of the very variable relationship between a ganglion in this position and the thyro-cervical trunk and its branches. One may, however, expect to find a branch either of the inferior thyroid artery itself or of the thyro-cervical trunk passing across the ganglion, either anteriorly or posteriorly, and contributing to the arterial net on its anterior surface by one or more ganglionic vessels. When, as is commonly the case, the ganglion

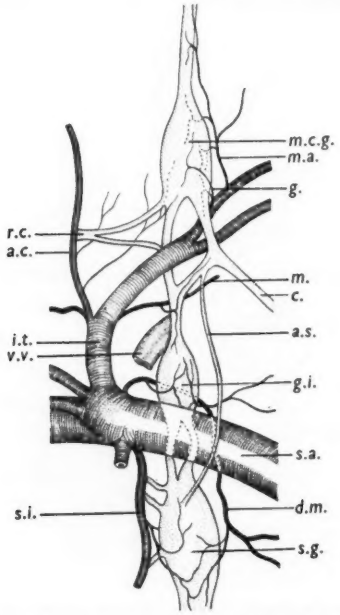


Fig. 9. Drawing from a dissection of the lower part of the right cervical sympathetic trunk. The trunk divided behind the subclavian artery, embracing the vertebral artery, the inferior cervical ganglion being incorporated with the first thoracic ganglion. *d.m.* descending mediastinal branch of thyro-cervical trunk; *g.* direct ganglionic branch of inferior thyroid artery; *m.* muscular branch; *s.a.* subclavian artery; *s.g.* stellate ganglion; *s.i.* superior intercostal artery.

lies on the medial side of the thyro-cervical trunk, the vessel concerned passes in a medial direction (Figs. 8, 9), and, having provided the ganglionic vessels, divides into branches that supply the longus cervicis and the oesophagus. In several cases this artery was a relatively large vessel and sent a branch downwards on the medial side of the sympathetic trunk, passing behind the subclavian artery and entering the thorax, where it gave branches to the oesophagus and adjacent mediastinal structures. When present this mediastinal vessel very commonly contributes one or more branches to the first thoracic or stellate ganglion from the medial side (Fig. 9).

When the thyro-cervical trunk arises more medially from the first part of the subclavian artery and lies at some distance from the medial border of scalenus anterior, the ganglion may be placed posterior to it or even towards its postero-lateral side. In this position the ganglion commonly receives its blood supply from a muscular branch of the inferior thyroid artery that runs laterally towards the scalene muscles. It may also receive an ascending branch from the costo-cervical trunk.

In spite of the intimate relationship that exists between a low middle cervical ganglion or a ganglion intermédiaire and the vertebral artery, in no case did the latter contribute to the ganglionic blood supply.

DISCUSSION

Other workers who have studied the sources of arterial supply to the superior cervical ganglion have been unanimous in regarding the ascending pharyngeal artery as the most constant and important source. Varying statements have been made in regard to the named branches of this artery that contribute the ganglionic vessels, but little detailed description is available. De Souza (1932) noted short medial ganglionic branches arising from the trunk of the artery, but did not correlate their presence with the level of origin of the parent vessel from the external carotid artery. He also stressed the prevertebral branch that courses down the lateral border of the ganglion, providing ganglionic branches that ramify on the anterior surface. The present reinvestigation substantiates the importance of this fairly constant laterally situated vessel.

With regard to the other arteries that supply the ganglion, de Souza regarded the superior thyroid artery as a constant source and mentioned the origin of the ganglionic branch from a point near the commencement of the parent vessel and its recurrent course. Other workers, notably Delamere & Tanasesco (1906), mention the superior thyroid artery as a variable source of supply only. The writer's observations, though conforming with the latter view, especially in regard to the variation in the size of the contribution from this artery, support de Souza to the extent of confirming that the superior thyroid artery is a fairly constant source.

The inferior thyroid artery has not been regarded by other investigators as playing a part in the arterial supply of the superior cervical ganglion, except that de Souza noticed branches rather rarely from the ascending cervical artery. The present re-examination of the matter indicates that the ascending cervical artery quite frequently supplies the lower portion of the ganglion and that, even when it does not do so, a longitudinal vessel from the inferior thyroid trunk is commonly present, ascending on the chain to reach the ganglion.

Ganglionic branches arising directly from the external or common carotid artery are mentioned as rarities by de Souza (1932) and Bartholdy (1897), and as inconstant vessels by Delamere & Tanasesco. The writer has seen a comparable branch from the internal carotid artery, but not from the external or common carotid.

In regard to the middle cervical ganglion, it is clear that previous workers have not always paid sufficient attention to the influence of its variable position on its blood supply. Moreover, there appears to have been occasional confusion between a ganglion intermédiaire and the inferior cervical ganglion. The ganglion illustrated by

Delamere & Tanasesco, for instance, and labelled as the inferior cervical ganglion, is undoubtedly a ganglion intermédiaire. It is therefore sometimes difficult to interpret the reports of the blood supply to these ganglia.

The present investigation supports the observations of Lobstein (1823), Bartholdy, Delamere & Tanasesco, Anserow (1925) and de Souza on the constancy and importance of the supply to the middle cervical ganglion from the inferior thyroid artery. The ganglionic vessels from the ascending muscular branch of this artery, situated on the medial side of the sympathetic trunk and regarded by de Souza as the main source of supply, are confirmed. They are, however, only present when the ganglion is situated above the inferior thyroid artery. The variable contribution from the ascending cervical artery, mentioned by de Souza and by Delamere & Tanasesco, is also substantiated, but again only in a high position of the ganglion. In no case did the ganglion receive vessels from the inferior laryngeal artery, noted as a variable source by Delamere & Tanasesco. A contribution from the deep cervical artery noted by Bartholdy, although not seen in the present series, is no doubt similar to that from the costo-cervical trunk which is occasionally present when the ganglion is in a low position.

SUMMARY

1. The superior cervical ganglion receives its blood supply from several sources: (*a*) the ascending pharyngeal artery, the principal and only constant source. The arrangement of the ganglionic arteries arising from its prevertebral branches, meningeal branch and the parent trunk are described; (*b*) the superior thyroid artery, a very common but inconstant source, the extent of its contribution being somewhat variable; and (*c*) the inferior thyroid artery, which commonly provides ganglionic branches either through its ascending cervical branch or by a longitudinal vessel ascending on the sympathetic trunk.

Less commonly the occipital and internal carotid arteries provide a supplementary blood supply.

2. The middle cervical ganglion shows variations in the pattern of its ganglionic vessels according to its relation to the loop of the inferior thyroid artery, its principal and often sole source of supply. The usual arrangement of these vessels in high and low positions of the ganglion is described. In the low position, the thyro-cervical and costo-cervical trunks may contribute to its arterial supply.

3. The ganglion intermédiaire receives branches similar in source and arrangement to those of a low middle cervical ganglion.

I wish to express my gratitude to Prof. G. A. G. Mitchell for his continuing interest in this work and for much helpful advice, willingly given on so many occasions. I would also gratefully acknowledge the valuable assistance I received from Mr H. Gooding and his assistants on the technical staff of the Department. A part of the expenses connected with the investigation has been defrayed by a departmental grant from the Medical Research Council.

REFERENCES

ADAMS, W. E. (1942). The blood supply of nerves. I. Historical review. *J. Anat., Lond.*, **76**, 323–341.

ANSEROW, N. I. (1925). Die Arterien des Rückenmarks, der Intervertebral-ganglien und der sympathischen Ganglien. *Anat. Ber.* **3**, 348–349.

BARTHOLDY, K. (1897). Die Arterien der Nerven. *Morph. Arb.* **7**, 393–458.

DELAMERE, G. & TANASESCO, J. C. (1906). Étude sur les artères du sympathique céphalique, cervical, thoracique et abdominal. *J. Anat., Paris*, **42**, 98–107.

DE SOUZA, O. M. (1932). Contribution à l'étude de la vascularisation du système nerveux organo-végétatif. *Ann. Anat. path. méd.-chir.* **9**, 975–997.

LOBSTEIN, J. F. (1823). *De Nervi Sympathetici humani fabrica usu et morbis*, pp. 67–68. Paris: Levrault.

THE RELATIONSHIP BETWEEN INTERNODAL LENGTH AND GROWTH IN HUMAN NERVES

By A. D. VIZOSO
Department of Anatomy, University College, London

INTRODUCTION

It has been shown in a number of animal species that the length between the nodes of Ranvier of peripheral nerve fibres increases with the diameter of the fibre (Key & Retzius, 1876; Boycott, 1904; Takahashi, 1908; Hatai, 1910; Kubo & Yuge, 1938; Hiscoe, 1947; Thomas & Young, 1949). Further, it is known that the slope of the line expressing this relationship is related to the growth of the part in which the nerve lies (Vizoso & Young, 1948). When a nerve regenerates in an adult animal, that is to say an animal no longer growing, the internodal length does not return to its original measurements but remains constant throughout the whole range of fibre diameters, even after the fibres have again reached their normal size (Young, 1945; Hiscoe, 1947; Vizoso & Young, 1948).

If internode length is so closely related to growth it should be possible to reveal differences between the nerves of parts of the body that grow to different extents. Such differences of growth of parts are very marked in man, and Shephard, Sholl & Vizoso (1949), in particular, have shown that the jaw grows very much less in absolute length than either the forearm or the leg in the period from birth to 20 years of age. The present paper records a study of internodal length in nerves taken from these three parts of the body. The nerves used were the anterior tibial at the level of the ankle joint, the ulnar at the level of the wrist joint and the facial immediately proximal to its entry into the parotid gland.

METHOD

The material used consisted of various sets of nerves taken in the post-mortem room as soon after death as circumstances permitted. The time between death and the removal and fixation of the specimens varied from 4 to 23 hr. Stretches of nerves (3–4 cm. in the case of the anterior tibial and ulnar, and 1 cm. approximately for the facial) were carefully placed on small cards and then fixed in 4 % formaldehyde in 0·9 NaCl, in which they were left until required. On removal from this solution the material was stained in 1 % osmium tetroxide in 0·9 NaCl. An alternative procedure was to fix and stain the specimens directly with 1 % osmium tetroxide in a solution of 0·9 NaCl, and then to store the specimen in 30 % alcohol until required.

Before the fibres could be dissociated it was necessary to place the nerves in water with glycerine (one-third of glycerine by volume) for several days. This tends to counteract the brittleness which results after the nerves have been in osmium tetroxide solution.

The remainder of the technique employed has been described already by Vizoso & Young (1948). The methods of measurement of the fibres, treatment of the data,

effects of fixation and dehydration, the mechanical effect of removing fibres, and errors of measurement were also dealt with in that paper.

RESULTS

(1) *Nine-year-old female*

Material from the anterior tibial and facial nerves of a girl 9 years old who died of peritonitis was measured. In Fig. 1. the lengths of individual internodal segments from the anterior tibial nerve are shown plotted against their diameters. The fibres

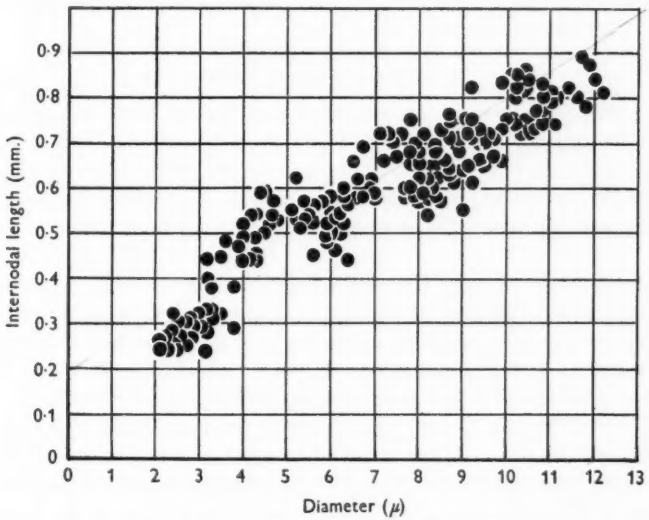


Fig. 1. Fibres from the anterior tibial nerve of a 9-year-old girl. Each point represents a single internode.

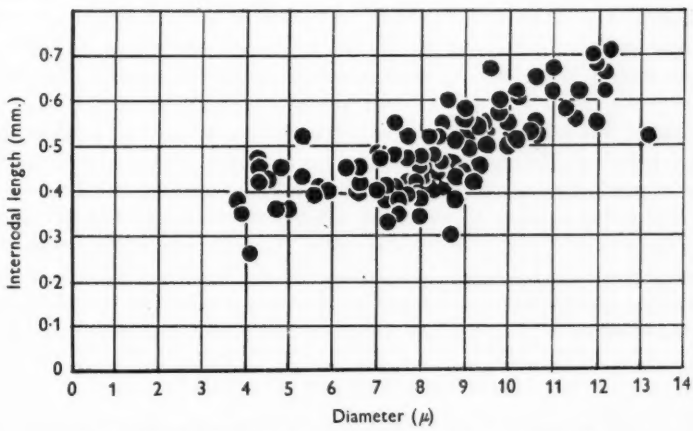


Fig. 2. Single internodes from the facial nerve of a 9-year-old girl.

measured range in diameter from 2 to 12μ and in internodal length from 0·25 to 0·85 mm. There is a direct relationship between diameter and internodal length (Fig. 1), but some of the fibres of the 3μ group and most in the 4μ group appear to fall above the line observed for the other groups. A greater degree of scatter is found in the observations for fibres of larger diameter.

Fig. 2 shows individual internodes for the facial nerve of the same subject. Unfortunately, there were few fibres recorded below 4μ in diameter, and none at all in the 2μ group. The scatter is larger than observed for the anterior tibial, and is most apparent in the 7–9μ group. The direction of the line suggested by the points is not as clear as it was for the anterior tibial, and a tendency to depart from linearity around the 7 and 8μ groups is visible.

(2) *Eighteen-year-old female*

Fig. 3 shows the individual internodes plotted against their corresponding diameters for the anterior tibial nerve of a girl 18 years old who died of pulmonary tuberculosis and diabetes. The trend suggested by the points is very similar to that in Fig. 1 for the 9-year-old girl; there is a suggestion of a kink in the line here, as in the previous case, but it appears at a higher level, among diameters of 5, 6 and some of 7μ.

In Fig. 4 the mean average internodal length is plotted against the mean fibre diameter in 1μ groups, ranging from 3 to 12μ. Comparing this line with that for the anterior tibial in Fig. 1 it will be seen that there is a kink present in both cases; in groups 3, 4 and 5μ in Fig. 1 and groups 5, 6 and 7μ in Fig. 4. The lines of the two graphs are similar, but in Fig. 4 there is a suggestion of a steeper rise.

Fig. 5 gives readings for the ulnar nerve of the 18-year-old girl. The range of fibre diameter extends to 13μ, and the internodal length to 1·1 mm. As in the anterior tibial, the relationship between diameter and internodal length is well marked. There seems to be a sudden increase in internodal length between the 4–5 and 5–6μ groups. The rate of increase of internodal length per diameter group appears to be somewhat less rapid above the 7μ diameter group than in the smaller ones. The grouped data (Fig. 4) show the sudden increase in internodal length more clearly. The results for the third nerve of this set, the facial, are shown in Fig. 6, where individual internode lengths are plotted against the corresponding diameters. The diameter range extends to the 13–14μ group. Although the scatter is probably wider than for the ulnar nerve, the majority of the readings fall close to the main line suggested by all the observations. There is no visible kink or step in the line but the slope is markedly less steep than in the ulnar and anterior tibial nerves.

The relationship existing between the different nerves studied in the 18-year-old subject can be seen in Fig. 4. From a common starting-point the anterior tibial and the ulnar nerves have a similar slope of line but the facial has a much more slowly rising gradient. It should be noted that a suggestion of a kink in the slope of the line for the facial nerve at the 5μ diameter group depends on a single observation.

(3) *Sixty-seven-year-old female*

In Fig. 7 individual internode length measurements are plotted against diameter values for the anterior tibial nerve of a woman 67 years old who died from a

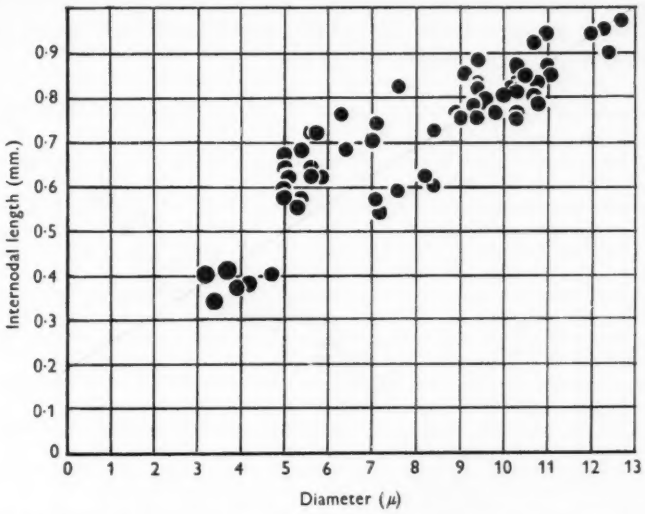


Fig. 3. Single internodes from the anterior tibial nerve of an 18-year-old girl.

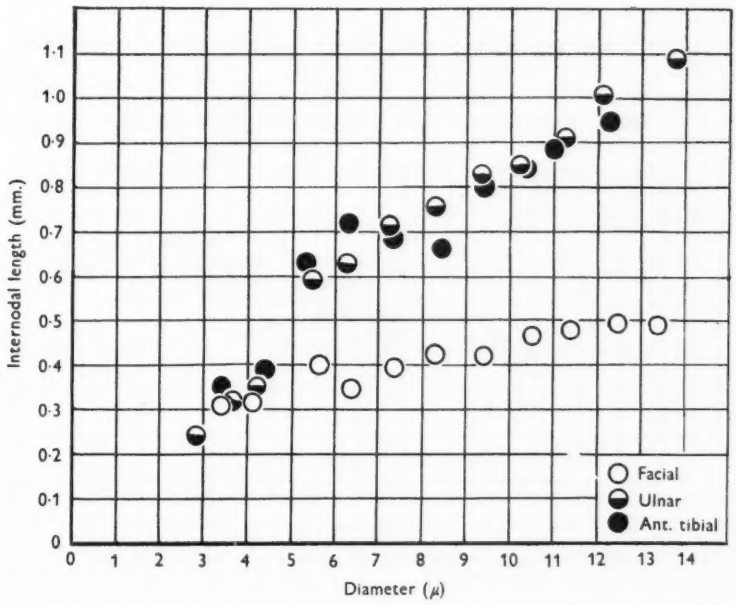


Fig. 4. Three sets of points representing mean values for 1 μ diameter groups for the anterior tibial, ulnar and facial nerves of an 18-year-old girl.

strangulated femoral hernia. This is clearly somewhat different from previous graphs for this nerve. First, the degree of scatter is appreciably larger; secondly, although the range of fibre diameter is the same as for younger individuals greater internodal lengths are observed, many being over 1·2 mm.

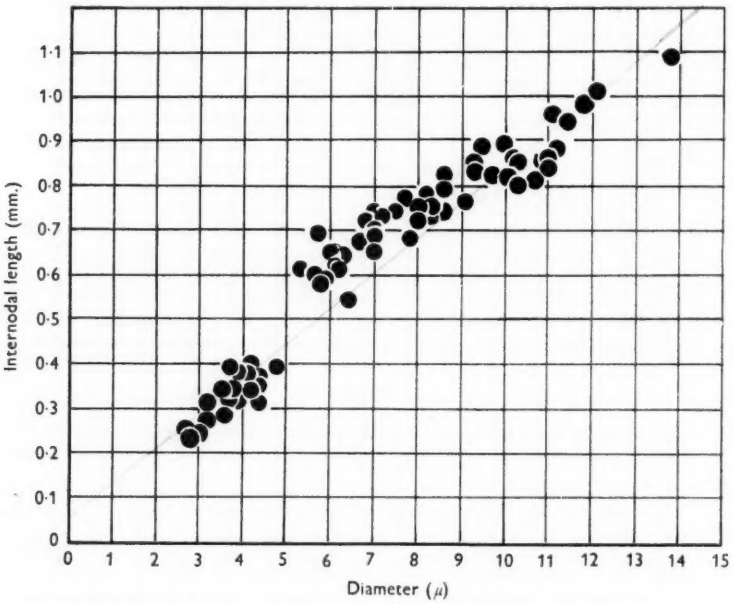


Fig. 5. Single internodes of the ulnar nerve of an 18-year-old girl.

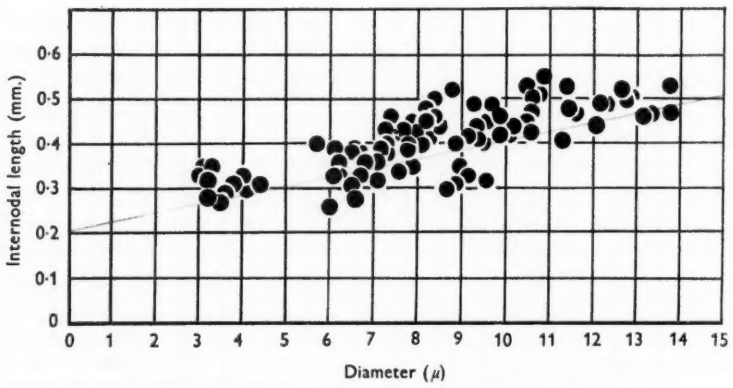


Fig. 6. Single internodes of the facial nerve of an 18-year-old girl.

These long internodes were mostly found in fibres of 11 μ or more in diameter. Still more striking is the fact that there are a number of relatively short internodes in

the 6–11 μ range, suggesting a second trend in the data. The slope of the line is somewhat steeper than in the anterior tibial nerve of the 18-year-old girl.

Results from the ulnar nerve of this 67-year-old woman are plotted in Fig. 8. Although there are less observations here than for the anterior tibial nerve a fair amount of scatter is apparent. The upwards trend is close to linearity, but as in the anterior tibial there are some short internodes in the 6–10 μ diameter groups.

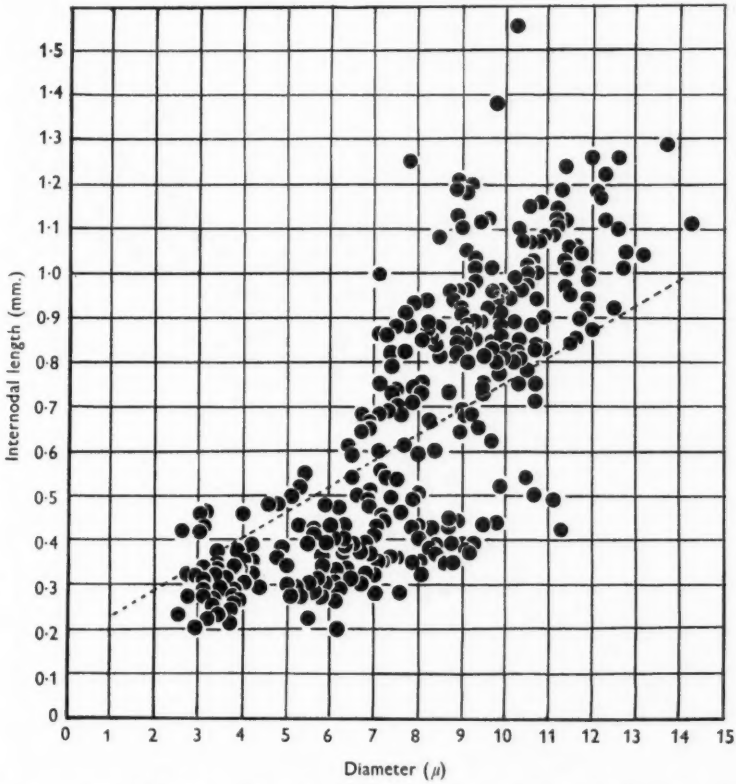


Fig. 7. Single internodes of the anterior tibial nerve of a 67-year-old woman. The broken line corresponds to an hypothetical line calculated from initial internodal length measurements and the size of the lower leg (see p. 352).

(4) *Eighty-year-old male*

Results from the fibres of the anterior tibial nerve of a man 80 years old who died of cerebral injury are plotted in Fig. 9 as single internodal length observations against corresponding fibre diameters. The picture obtained is strikingly different from those of younger subjects, and even from that of the 67-year-old woman.

The line starts with diameter readings of about 4 μ corresponding to internodal lengths shorter than any recorded, even for the thinner fibres of younger subjects.

A very steep rise leads to readings for internodal length of 1·1–1·2 mm. in diameter groups ranging from 8 μ. The scatter is large and may be compared with that for the anterior tibial nerve at 67 years of age in Fig. 7.

The ulnar nerve of this old individual, shown in Fig. 10, shows similar conditions. The degree of scatter is large. The line relating internodal length to diameter is less steep than that for the anterior tibial, especially after the 13 μ group. However, in view of the great scatter the grouped results do not adequately express the relations found.

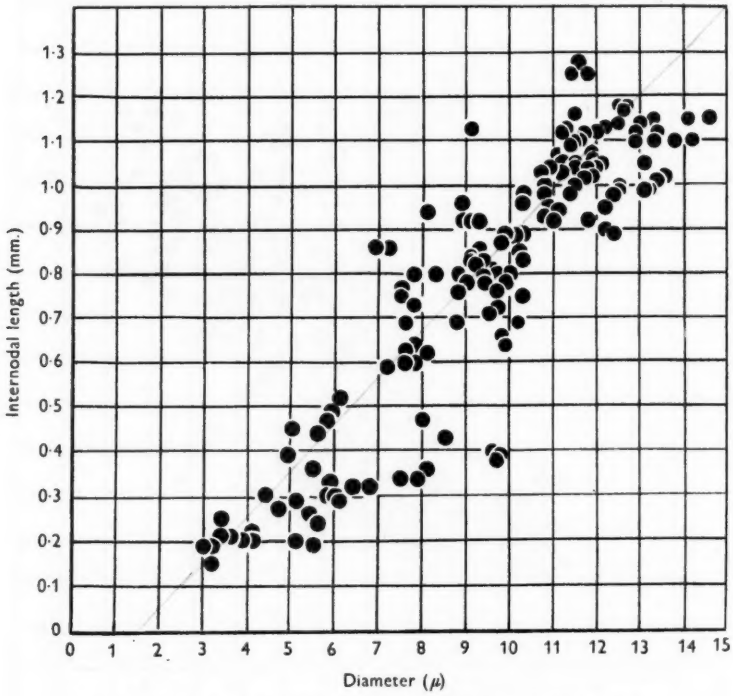


Fig. 8. Single internodes of the ulnar nerve of a 67-year-old woman.

The facial nerve of the 80-year-old male provides the data for Fig. 11. Here individual measurements show a clearer trend of increase than in the ulnar and anterior tibial nerves.

In so far as conclusions can be drawn from the plots of the grouped data they show that the differences found in younger subjects between slopes of the lines for the facial and other two nerves have disappeared. However, a better way of expressing the result is to say that in older subjects in the nerves of the limbs, but not of the face, many of the larger fibres have relatively shorter internodes than in younger subjects.

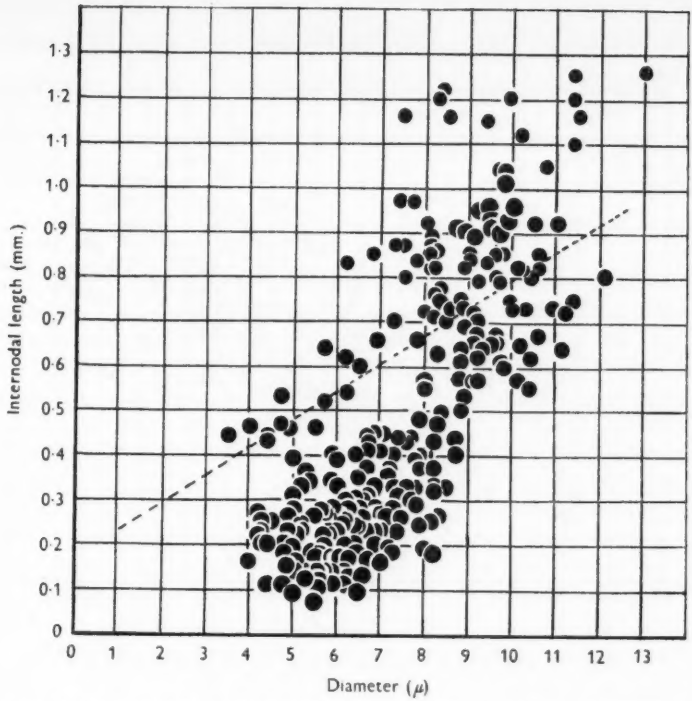


Fig. 9. Single internodes of the anterior tibial nerve of an 80-year-old man. Hypothetical line calculated as in Fig. 7.

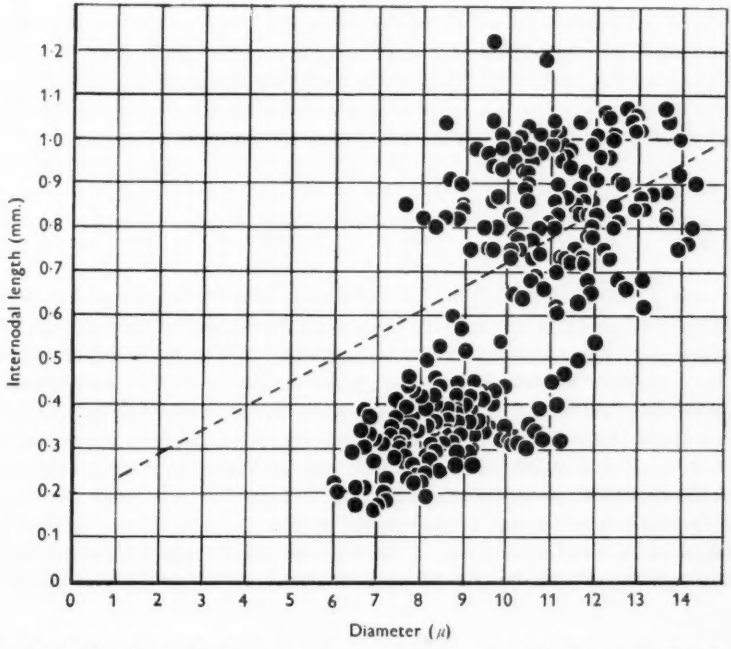


Fig. 10. Single internodes of the ulnar nerve of an 80-year-old man. Dotted line as for Figs. 7 and 9.

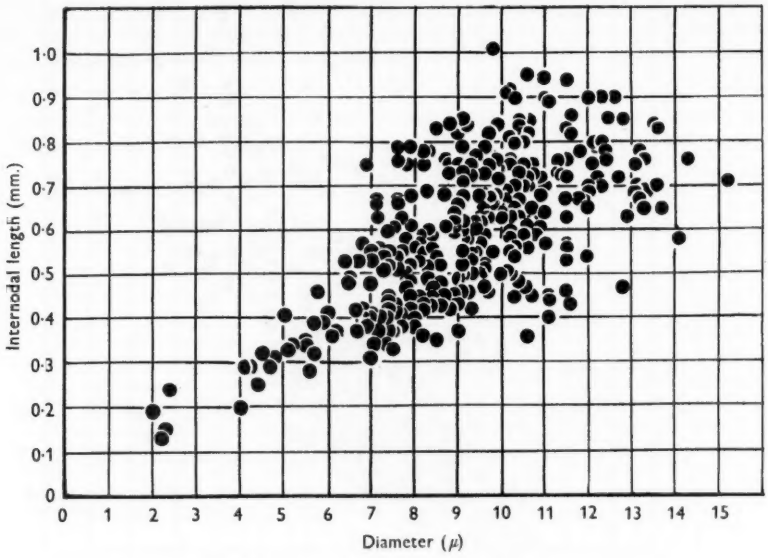


Fig. 11. Single internodes of the facial nerve of an 80-year-old man.

DISCUSSION

(1) *Increase of internode length with growth*

The data of the various nerves from the individual 18 years old clearly support in a general way the thesis that internode length increases with diameter faster in nerves lying in the parts of the body that have grown the more in length since birth. From the data collected by Shephard *et al.* the following figures have been obtained by finding the mean of all newborn and 18-year-old subjects measured.

	Birth (mm.)	18 years (mm.)	Increase (mm.)
Lower leg	130	522	4·0 ×
Forearm	139	462	3·3 ×
Jaw	59	119	2·0 ×

The nerves examined lie within the parts as defined by Shephard *et al.* and were therefore stretched approximately to the extent shown in the third column above. We cannot say exactly what increase has occurred in any given internode length, since the condition at birth is unknown in man. However, it is not unreasonable to suppose that the ultimately largest nerve fibres in each nerve will have medullated by the time of birth (Cottrell, 1940). The spacing at the nodes in a newborn rabbit was given by Vignal (1889) at about 250 μ, and by Vizoso & Young (1948) at 250–300 μ. Hiscoe (1947) gives 300 μ in the rat. The shortest internodes found in the 9- and 18-year-old individuals in the present study are about 235 μ, though the older individuals show shorter internodes. The data therefore suggest that the nodes are first laid down at a periodicity somewhat less than 250 μ—possibly at about 230 μ.

If this is so we have for the longest internodes of the 18-year-old subject the following figures:

	Birth (presumed) (μ)	18 years (μ)	Increase (μ)
Anterior tibial	230	980	4·3 ×
Ulnar	230	1100	4·8 ×
Facial	230	550	2·4 ×

There is clearly a general agreement with the relative increases in the length of the parts given above. The nodes appear to have separated slightly more than the bones have grown, but it must be remembered that the figures are for the whole parts and do not indicate the exact amount of growth for the region of nerve examined. The ulnar internodes have grown more than expected, but this appearance depends on the two very long internodes shown in Fig. 5. Exact correspondence with the growth figures can in any case hardly be expected, since the subject was a girl and the data of growth rate were for boys. Considering the known sources of error the results agree with the view that the internode length is related to the growth of the nerve.

Ranvier (1875) was probably the first to realize that the increase in internodal length is related to the increase in size of the part in which the nerve lies. He compared the length of the segments in the newborn and adult dog, and found that it increased from 0·3 mm. to 1·3 mm. He then stated that the increases in the length of the nerves appeared to be due to the progressive elongation of their internodal segments.

This connexion was denied by Vignal (1889), but Young (1945) and Vizoso & Young (1948) have shown that it is supported by many further facts, for instance the slope of the line relating internode length to fibre diameter increases with the growth of the limb. Thomas & Young (1949) have produced further evidence by showing that in fishes the lateral line nerves have much longer internodes than have the branchial nerves.

The present study shows an exactly similar relationship in man, with the added advantage that the growth of the parts has been studied exactly. The factors that determine internode length may be complex, but there is no doubt that the amount of increase in length of the nerve subsequent to medullation is a very important factor.

(2) *Changes of internodal length in old age*

The evidence so far considered shows that the number of nodes on a fibre remains nearly constant in the earlier decades of life. The data from older people show that there is later a multiplication of nodes, presumably due to degeneration and reformation. The scatter of internode lengths for a given fibre diameter is greater in the 67-year-old, and especially in the 80-year-old subject, than in the one of 18. Data such as those of Fig. 7 suggest that we have two populations of internodes, those which have remained with the individual throughout life, and others which have formed later, after the growth period was over, and therefore remain close to the minimum length. Internodes of this other set are similar to those found in regenerated nerves of adult rabbits and rats by Young (1945), Hiscoe (1947) and Vizoso & Young

(1948), in that they are short, even on fibres of considerable diameter. Such new nodes might be formed on fibres which had either undergone complete degeneration and regeneration, in which case all segments are short, or have been formed between two retracted nodes. Probably both of these processes go on. Among those fibres that have retained the original internode length, there are some which have decreased in diameter (Figs. 7, 9 and 10) and have shifted towards the left on the graph, giving the appearance of a steeper slope than the one expressed by the line for a young adult. When the fibres continue to lose their diameters either the internodal segments may become unstable and divide into smaller segments or the fibres degenerate. The broken line in Figs. 7, 9 and 10 represents the hypothetical length/diameter relationship for a normal young adult having approximately the same limb measurements as the subject. It has been fitted through two points, one at 1 μ diameter, with the minimum internodal length 0·230 mm.; the other is the product of the original internodal length (0·230 mm.) by the number of times the limb has grown since birth for the maximum diameter. It is clear that these lines do not fit the data, that is to say that the process of ageing introduces a new factor in the control of internode length. Renaut (1881) described short segments in the nerves of old horses and donkeys, and suggested that they were new ones produced to replace elements 'dont l'évolution est terminée'.

There is some evidence that when there is a local degeneration the neighbouring segments may extend, for it is noticeable that the nerves of the old individuals, besides having many short internodes also show some that are longer than any found in the younger subjects.

Some of the internodes found in these old individuals are actually shorter than any found at younger ages. This may be due to a change in the nature of the myelin, producing a shorter minimum periodicity.

SUMMARY

1. The length of the internodes was measured in human nerves taken from parts of the body that differ in amount of post-natal growth.

2. In a subject 18 years old internode length was found to increase with fibre diameter faster in the ulnar and anterior tibial nerves than in the facial.

3. The increase of length of the largest internodes was estimated to be 4·3 × in the anterior tibial nerve, 4·8 × in the ulnar, and 2·4 × in the facial, the increases in the length of the corresponding parts being 4·0, 3·3 and 2·0 times. Internode length is therefore related approximately to growth.

4. In a subject 9 years old the increase of internode length with fibre diameter was less steep than in the 18-year-old, and there was less difference between the nerves from fast- and slow-growing regions.

5. A subject 67 years old showed some fibres with internodes unduly short relative to their diameter and these were still more numerous in a subject of 80. These short internodes presumably lie on fibres that have undergone either local demyelination or complete Wallerian degeneration and regeneration.

My grateful thanks are due to Prof. J. Z. Young for his advice and criticism, to Prof. G. R. Cameron for giving me access to material, to Dr R. H. Shephard for his

help at the beginning of this work and also to the Council of the Royal Society for providing a grant from the Head Fund for maintenance during the period of the work.

The detailed data have been deposited in the Thane Library of the Faculty of Medical Sciences, University College, London.

REFERENCES

BOYCOTT, A. C. (1904). On the number of nodes of Ranvier in different stages of growth of nerve fibres in the frog. *J. Physiol.* **30**, 370–380.

COTTRELL, L. (1940). Histologic variations with age in apparently normal peripheral nerve trunks. *Arch. Neurol. Psychiat., Chicago*, **43**, 1138–1150.

HATAI, S. (1910). On the length of internodes in the sciatic nerve of *Rana temporaria* and *Rana pipiens*. *J. comp. Neurol.* **20**, 19–47.

HISCOE, H. B. (1947). The distribution of nodes and incisures in normal and regenerated nerve fibres. *Anat. Rec.* **99**, 447–475.

KEY, A. & RETZIUS, G. (1876). *Studien in der Anatomie des Nervensystems und des Bindegewebes.* Stockholm: Sampson and Wallin.

KUBO, M. & YUGE, A. (1938). *Collected papers: tribute to Professor Isikawa in celebration of his 60th birthday*, p. 114. Kyoto, Japan.

RANVIER, C. (1875). *Traité technique d'Histologie.* Paris: F. Savy.

RENAUT, M. J. (1881). Recherches sur quelques points particuliers de l'histologie des nerfs. *Arch. Physiol. norm. path.* **8**, 161–190.

SHEPHARD, R. H., SHOLL, D. & VIZOSO, A. D. (1949). The size relationship subsisting between body length, limbs and jaw in man. *J. Anat., Lond.*, **83**, 296–302.

TAKAHASHI, K. (1908). Some conditions which determine the length of internodes found on the nerve fibres of the leopard frog, *Rana pipiens*. *J. comp. Neurol.* **18**, 167–197.

THOMAS, P. K. & YOUNG, J. Z. (1949). Internodal length in nerves of fishes. *J. Anat., Lond.*, **83**, 336–350.

VIGNAL, W. (1889). *Développement des éléments du Système nerveux cérébro-spinal.* Paris: Masson.

VIZOSO, A. D. & YOUNG, J. Z. (1948). Internode length and fibre diameter in developing and regenerating nerves. *J. Anat., Lond.*, **82**, 110–134.

YOUNG, J. Z. (1945). History of the shape of a nerve fibre. (From *Essays on Growth and Form*, presented to D'Arcy W. Thompson.) Oxford: Clarendon Press.

AN EXPERIMENTAL STUDY OF THE FORNIX IN THE RABBIT

By JAMES M. SPRAGUE* and MARGARET MEYER
Department of Human Anatomy, University Museum, Oxford

The extrinsic connexions of the hippocampus have been studied in a number of vertebrate types, and the complexity of the system has been described by many workers (cf. von Kölliker, 1894, 1896; Elliot Smith, 1896; Cajal, 1911; Johnston, 1913; Kappers, Huber & Crosby, 1936; Young, 1936). The voluminous and controversial literature based on normal material has been reviewed by these authors and will not be repeated here. The experimental papers are few (cf. Edinger & Wallenberg, 1902; Gudden, 1881; Probst, 1901; Allen, 1944; Fox, 1943; Gerebtzoff, 1941–2), and these are based chiefly upon the Marchi method and, to a lesser extent, upon studies of retrograde cell degeneration. The information thus gained, however, is incomplete and contradictory, and there is thus need to undertake a re-examination of this system with modern methods.

The present study reports the results of experiments in rabbits in which small lesions have been placed in various parts of the fornix system (Text-fig. 1), the brains being subsequently prepared by a modification of the silver method of Glees (1946). This technique demonstrates in very satisfactory fashion the degenerative fragmentation of nerve fibres and their terminals, whether the latter consist of boutons terminaux or a pericellular plexus (free endings). The terminals may show marked degenerative changes as early as the third day following section of the axons, though in the case of the larger fibres these may not be apparent until the fifth day. During this period the degeneration is wholly distal to the site of the lesion with the exception only of retraction bulbs which are developed at the cut ends of the fibres. The method forms, without question, the most reliable, sensitive and precise technique for the study of fibre degeneration following axonal interruption within the central nervous system which is available at present, although isolated fibre connexions of minor significance are difficult to follow and may be overlooked.

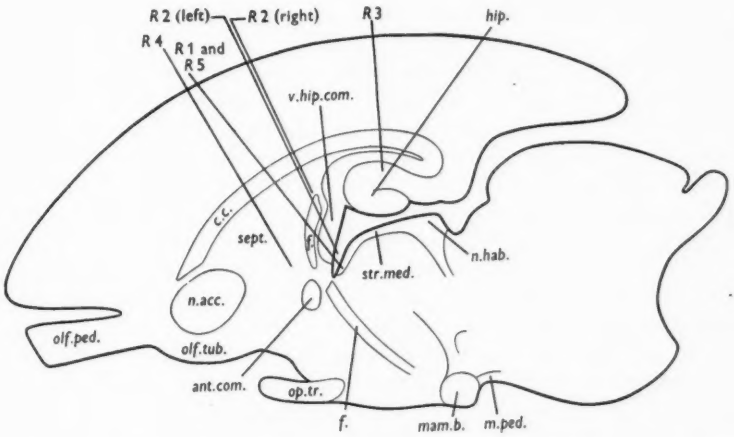
The fornix represents the only known efferent pathway of the hippocampus. It arises, according to Allen (1944), from all the pyramidal cells of the hippocampus and also from isolated pyramidal cells of the polymorph layer of the dentate gyrus. Fibres leaving the hippocampus collect in the alveus to form the large tract known as the fimbria, and the latter splits into two components, commissural and projection. The projection fibres have been divided into those which pass in front of the anterior commissure into the septum (precommissural fornix), and those which form the descending column of the fornix (or postcommissural fornix) and terminate mainly in the mammillary body. In normal, and in some experimental studies it has also been stated that fornix fibres have been traced to other centres such as the preoptic

* John Simon Guggenheim Memorial Foundation Fellow. On leave of absence from the Department of Anatomy, The Johns Hopkins University, Baltimore, Maryland, U.S.A.

region, to hypothalamic nuclei other than the mammillary body, to the habenula, and even into the tegmental region.

The commissural fibres connect the hippocampi of the two hemispheres. In the rabbit a differentiation into dorsal and ventral hippocampal commissures exists, described as the dorsal and ventral psalterium by von Kölliker (1896). According to Cajal (1911) the dorsal psalterium consists chiefly of the crossed temporo-ammonic tract and is therefore not primarily interhippocampal in its connexion. The ventral psalterium, on the other hand, is supposed to be a true commissure, though its exact terminations have not so far been determined experimentally.

Longitudinally orientated fibres which lie between the corpus callosum and the dorsal part of the hippocampus constitute another component of the fornix system.



Text-fig. 1. Outline of sagittal section through a rabbit's brain indicating the different positions of the lesions in the experiments described.

Considerable confusion in the terminology of this subcallosal part of the fornix exists in the literature, and it does not appear profitable to review the different usages of terms such as 'fornix of Forel', 'fornix longus', 'superior fornix' or 'dorsal fornix'. The term dorsal fornix is used here to designate the whole of this longitudinal system consisting, as Cajal suggested, of a variety of afferent and efferent pathways, including the fibres penetrating the corpus callosum, possibly fibres running forward from the dorsal psalterium, and afferent fibres said to arise in the nucleus of the diagonal band of Broca.

The experiments here recorded are intended to give a conclusive answer to a number of disputed points: (1) the termination of the fornix in respect of the different nuclei of the mammillary body; (2) the existence and relative importance of fornix fibres which end in other parts of the hypothalamus; (3) the existence and composition of fornix fibres which pass beyond the mammillary body into the tegmentum; (4) the possible contribution of any part of the fornix system to the habenula by way of the stria medullaris; (5) the septal termination of any part of the fornix system; (6) the

composition of the hippocampal commissures; and (7) the origin and termination of fibres of the dorsal fornix.

In all the cases described, fibres of the cingulum were severed and show degeneration. Since the origin and termination of the cingulum is outside the scope of this report, they will be the subject of a special study.

MATERIAL

Eight young rabbits were operated upon under the usual aseptic conditions with the use of pentobarbital sodium (Abbot) or of Dial (Ciba), administered intraperitoneally. A burr hole was made in the superior aspect of the parietal bone and slightly enlarged, the dura incised and the underlying cortex exposed. A narrow scalpel was then inserted vertically close to the median fissure and moved laterally through a distance of approximately 5 mm. in order to sever the fornix. The animals were killed 3–5 days after the operation by intravascular perfusion, under anaesthesia, of normal saline, followed by 10% formol-saline. The lesions were in each case checked histologically. It was found that in all cases the cingulum and the supracallosal striae had been cut by the incision, as well as the fornix. Cortical injury was practically confined to the immediately adjacent part of the cingulate gyrus. Interference with the vascular supply had, in some cases, resulted in an area of necrosis in the ventral hippocampal commissure, and this has been referred to in the description of the individual experiments. Frozen sections were cut and treated with silver, cresyl violet and Weil's stain. Selected sections were traced with a microprojector and the distribution of the degeneration plotted under a 2 mm. oil-immersion lens. Only the most representative sections from each case are recorded in the diagrams; many other levels were examined, and each case was studied independently by both authors. Five of the eight experimental animals are described; the remaining cases were used for confirmation of the results obtained.

RESULTS

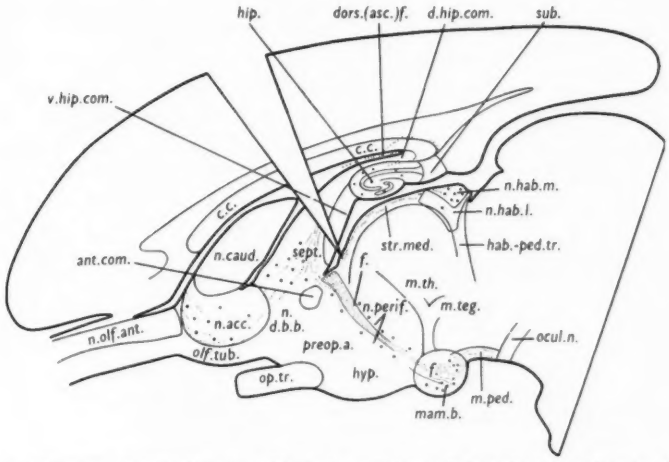
*R.*1, *survival time* 5 *days* (Text-fig. 2)*

The lesion on the right side cuts the fimbria and the dorsal fornix, injuring the septum and the stria medullaris; much of the ventral hippocampal commissure is destroyed. The lesion is similar but less complete on the left side. The brain was sectioned sagittally.

Anterior to the lesion on the right side, fibres of the fornix are degenerated. Some can be traced into the septal region; they terminate in the lateral and medial septal nuclei, but mainly in the antero-ventral part of the nucleus accumbens. All the remaining fibres of the fornix form the descending column which is completely degenerated. In traversing the hypothalamus, fibres terminate along the whole length of the perifornical nucleus surrounding the fornix. The other hypothalamic nuclei anterior to the mammillary body, and also the preoptic region, are normal. Within the mammillary body the degenerating fibres of the fornix form most of the posterior part of the capsule surrounding it, and they appear to spread anteriorly in

* In all the text-figures degenerating fibres are indicated by fine dotted lines and degenerating terminals by coarse dots.

the fibre capsule to terminate in the basal and intermediate parts of the medial mammillary nucleus (Pl. 1, fig. 2). Terminal degeneration is particularly severe in the basal area (Pl. 1, fig. 1), which is situated in the ventral and posterior part of the mammillary body.* Some fascicles of degenerating fibres overshoot the mammillary body and can be traced into the mammillary peduncle as far as the point of exit of the oculomotor nerve. The fine fornix fibres are easily distinguished among the heavy fibres of the peduncle. The more superficial fibres of the stria medullaris are degenerated and can be followed into the habenular nuclei. Terminal degeneration is chiefly confined to the medial habenular nucleus. Since the lesion in the ventral hippocampal commissure is extensive, there is severe fibre and terminal degeneration in all layers of the hippocampus, and also in the dentate gyrus.



Text-fig. 2. Diagram of sagittal section of brain in experiment R. 1, right side, indicating the lesion and the resultant fibre and terminal degeneration.

Some of the fibres of the dorsal fornix show fragmentation; they appear to penetrate the corpus callosum and, after joining the deep layers of the cingulum, curve round the splenium of the corpus callosum and apparently terminate in the hippocampus. Most of the fibres penetrating the corpus callosum are normal.

R. 2, *survival time* 5 *days* (Text-figs. 3, 4)

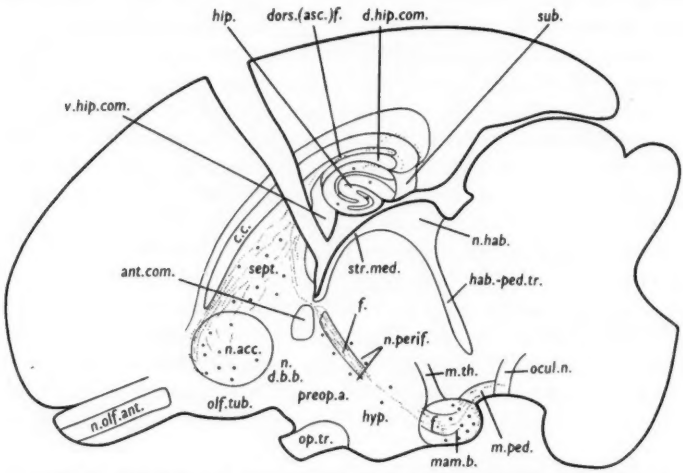
On the right side the fimbria is almost spared, the dorsal fornix has been cut and the posterior part of the septum is injured. On the left side the lesion is more extensive, apparently cutting the fimbria entirely. Injury to the ventral hippocampal commissure is slight, being confined to a local, clean-cut, incision in the medial part of the left side only. The stria medullaris has remained uninjured on both sides.

On the left side (Text-fig. 3), terminal degeneration in the septal nuclei is severe, and fascicles of degenerating fibres pass through the septum to collect in a compact

* Diagrams of transverse sections are shown in text-fig. 7 *a–c* to illustrate the distribution of degenerating fibres in the mammillary nuclei after interruption of the fornix.

subcallosal tract which is completely degenerated. This tract borders the superior and rostral surface of the nucleus accumbens, and many of its fibres terminate in this nucleus. It appears that some of these degenerating fibres turn laterally when reaching the medial forebrain bundle, but they could not be traced beyond this point. The olfactory tubercle, the nucleus of the diagonal band of Broca, the anterior olfactory nucleus and the preoptic region are intact. The descending column of the fornix passing through the septum is degenerated, while fibres which lie between the ventral hippocampal commissure and the descending column are normal.

Traversing the hypothalamus, the descending fornix column and the mammillary body present the same picture as that described for **R.1.** As in the latter case, a considerable number of degenerated fascicles overshoot the mammillary body into



Text-fig. 3. Diagram of sagittal section of brain in experiment R.2, left side.

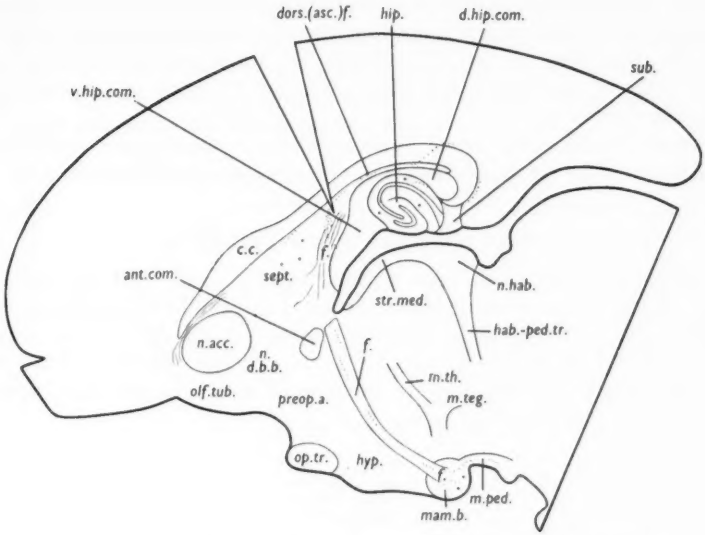
the tegmental region and can be traced among the fibres of the oculomotor nerve. The stria medullaris and the nuclei of the habenula are intact.

The area posterior to the lesion shows essentially the same degenerative changes as R.1, except for much less degeneration in the hippocampus which is explained by the small extent of the lesion in the ventral hippocampal commissure.

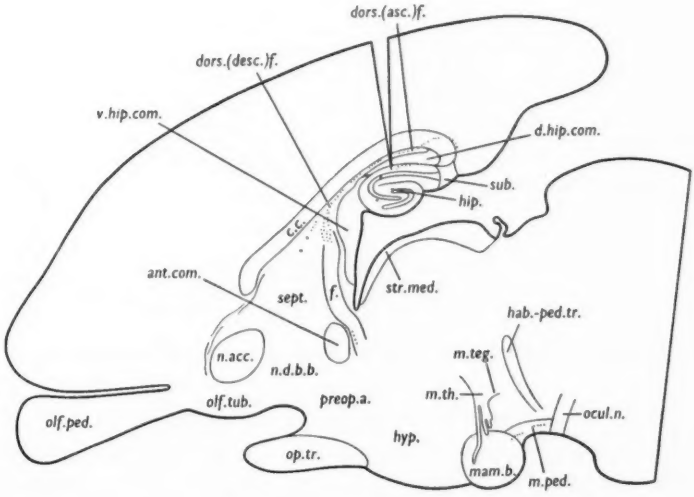
On the right side (Text-fig. 4), the septal degeneration is limited to a small amount in the lateral septal nucleus. Degenerating fascicles join the otherwise normal pillars of the fornix dorsally as it passes through the septum. There is little degeneration in the descending column of the fornix and in the mammillary body, but the fascicles passing into the tegmentum show quite severe degenerative change.

R.3, survival time 5 days (Text-fig. 5)

The lesion is entirely limited to the right side, cutting the dorsal fornix and only at one point penetrating into the hippocampus. There is no injury to the septum, fimbria or stria medullaris. Rostral to the lesion the fine fibre fascicles of the dorsal



Text-fig. 4. Diagram of sagittal section of brain in experiment R. 2, right side.



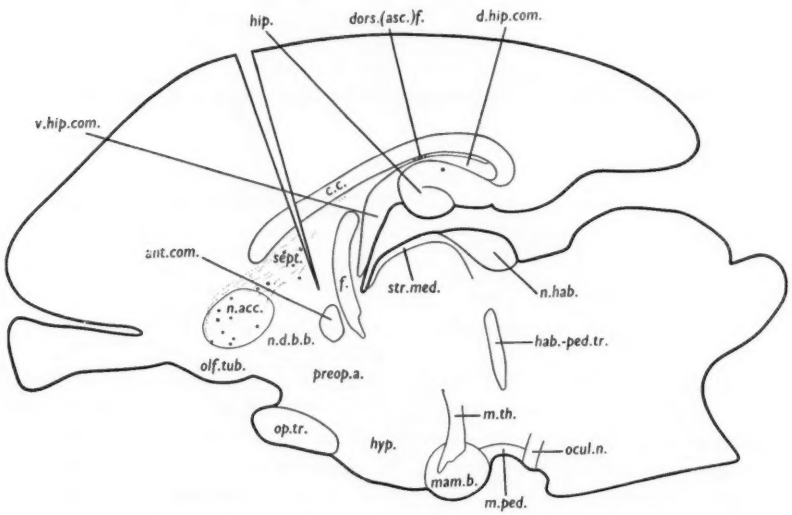
Text-fig. 5. Diagram of sagittal section of brain in experiment R. 3, right side.

fornix are completely degenerated. They are observed to turn ventrally into the upper part of the descending column of the fornix. Septal degeneration is very slight and confined to the lateral septal nucleus. There is slight degeneration in the lower part of the descending column of the fornix. In the mammillary body no change could be detected; on the other hand, a number of the fibres passing into the tegmentum are degenerated.

The degeneration in the dorsal fornix posterior to the lesion is similar to that already recorded for the other cases. The hippocampus shows little change.

R.4, survival time 5 days (Text-fig. 6)

The lesion is limited to the right septum, passing deeply into the lateral septal nucleus. Secondary degeneration in the septal nuclei and in the nucleus accumbens is severe and is similar to that in R.1 and on the left side of R.2. The descending column of the fornix, the mammillary body, the olfactory tubercle and the preoptic region are normal. The dorsal fornix posterior to the lesion and the hippocampus show very little change in this case.



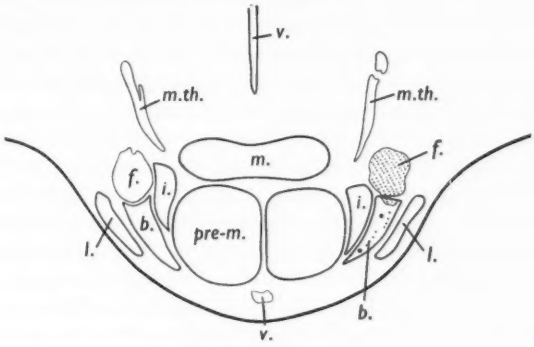
Text-fig. 6. Diagram of sagittal section of brain in experiment R.4, right side.

R.5, survival time 3 days (Text-figs. 7*a–c*)

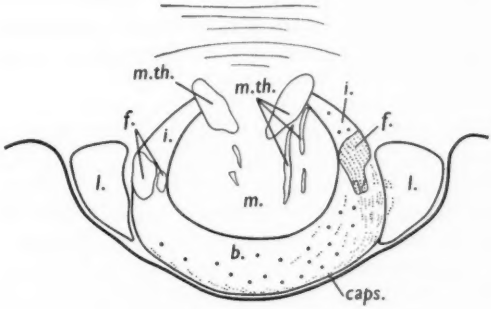
The lesion is entirely confined to the right side, cutting the fimbria and destroying most of the right half of the ventral hippocampal commissure. The posterior part of the septum and the stria medullaris have also been injured. The brain was cut transversely, and only a series of diagrams through the mammillary body is given to indicate the course and termination of fornix fibres within the nuclei of the mammillary body. The results relevant to other areas are not described, except to clarify points

which have remained doubtful from study of the sagittal sections of the other experiments.

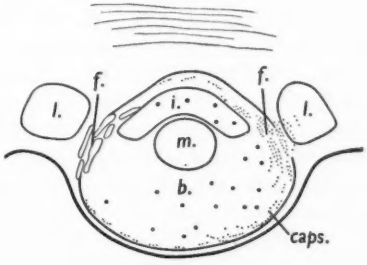
The right descending column of the fornix is degenerated. The contralateral side has been spared in this case, and the left descending column is entirely intact. The most medial fibres of the column of the fornix curving through the septum are normal on both sides, and are evidently ascending fibres.



Text-fig. 7*a*.



Text-fig. 7*b*.



Text-fig. 7*c*.

Text-figs. 7*a–c*. Series of diagrams of transverse sections through the mammillary body of experiment R. 5 to illustrate the distribution of degenerating fibres in the mammillary nuclei after interruption of the fornix.

In the anterior part of the mammillary body (Text-fig. 7*a*), there is no definite capsule. At this level the ventral part of the premammillary nucleus is seen and, superior to it, the anterior paired portion of the medial mammillary nucleus; the intermediate and basal parts are just beginning to appear. On the right very few degenerating fibres are seen in the basal part; the other nuclei on the right side and all those on the left are normal.

At the more caudal level shown in Text-fig. 7*b*, the degenerating fibres of the fornix are seen to contribute to the formation of the capsule of the mammillary body (fibres of the capsule which have other origins are intact). In the capsule degenerating fibres of the right fornix spread to the left side of the mammillary body. At this level there is terminal degeneration in the basal part of the medial nucleus, particularly in the proximity of the capsule; the intermediate part also shows a few degenerating terminals. Degenerating fascicles of the fornix spread out into the lateral nucleus, but they do not appear to end there; probably they rejoin the capsule more ventrally.

At the next level (Text-fig. 7*c*), the fornix is reduced to narrow bands of fibres which form most of the lateral part of the capsule. The intermediate nuclei have become fused in the midline and enclose the much decreased medial part of the medial mammillary nucleus. The basal part has expanded and, at a still more caudal level, it occupies the entire mammillary body. The distribution of the secondary degeneration in the different nuclei of the mammillary body is illustrated in Text-fig. 7*c*.

The right stria medullaris has been injured in this experiment, and the right medial habenular nucleus is filled with clusters of swollen, argentophil annular boutons (Pl. 2, fig. 3); in the nucleus of the left side the fibre plexus is normal (Pl. 2, fig. 4).

DISCUSSION

The different positions and the varying extents of the lesions in the experiments described are graphically summarized in Text-fig. 1. It will be observed that the projection systems of the fornix have been severed selectively or in different combinations, and the relation of the distribution of the resulting fibre degeneration to these variations in the lesions becomes readily apparent. Summarizing the results of each experiment, it is convenient to discuss parts of the fornix system separately.

Descending column of the fornix. The degenerating fibres of the descending column of the fornix were followed to their termination in three cases (R.1, R.2, R.5). These experiments provide no evidence that the hypothalamic nuclei which lie in front of the mammillary body receive any contribution from the fornix, with the exception of some fibres which leave the main bundle to terminate immediately in the perifornical nucleus. On the contrary, the experimental evidence shows that almost the entire descending column terminates in the latero-ventral and posterior parts of the medial mammillary nucleus. In lower mammals the medial mammillary nucleus is differentiated into a number of individual nuclei, which have been described in detail by M. Rose (1935) and J. Rose (1939–40). The silver method used in our experiments shows clearly that fibres of the fornix project only to the basal and intermediate parts of the medial mammillary nucleus* (J. Rose's terminology), and they terminate by means of a fine pericellular plexus which surrounds the nerve cells. The anterior and

* J. Rose finds that the basal and intermediate parts of the medial mammillary nuclei show the same cell type, and that both show a definite regression in higher mammals. However, it is not always possible to establish the homologies of these nuclei with accuracy in different mammalian groups.

medial parts of the medial mammillary nucleus which, according to the experimental work of van Valkenburg (1912), give rise to the mammillo-thalamic tract, have no direct connexion with the fornix.* Some fascicles of the fornix splay out into the lateral mammillary nucleus as has been described by Allen (1944) in Marchi material. Since we have found no evidence that fibres actually terminate in this nucleus, it is concluded that these fascicles may rejoin the capsule. The fornix enters the mammillary body at its dorso-lateral aspect, and much of the lateral and ventral, and almost the entire posterior, part of the capsule consists of fornix fibres. It may be noted that some authors (e.g. Allen, 1944) have held the view that the capsule is composed solely of fibres of the mammillo-thalamic tract.

It is confirmed in our material that fornix fibres do not cross the midline anterior to the mammillary body, which is in accordance with findings of Le Gros Clark (1938) and of Allen (1944). Only in the posterior part of the mammillary body is there a certain amount of overlap to the contralateral side by way of the capsule (Text-figs. 7*b*, *c*). Whether this can be interpreted as an illustration of bilateral representation is questionable, for in the rabbit's brain the mammillary bodies themselves are not bilateral as they are in higher mammals, and their nuclei in the posterior part are fused. It is probable, therefore, that this overlap does not exist in higher mammals where the two separate mammillary bodies are sharply divided by capsular fibres, and that in them the relationship between the hippocampus and the mammillary bodies is entirely unilateral.

A continuation of fornix fibres into the mesencephalon has been suggested by Edinger & Wallenberg (1902) and by von Kölliker (1896). In our experiments both the descending fibres of the fornix and the dorsal fornix contribute to a tract which descends in the fornix column and passes beyond the mammillary body into the mesencephalon, intermingling with fibres of the mammillary peduncle. These fibres are fine and can easily be distinguished from the coarse (undegenerated) fibres of the mammillary peduncle. In the material described they could not be followed farther than is indicated in the diagrams. Gerebtzoff (1941–2), however, described a component of the fornix terminating in a distinct nucleus of the bulbar reticular formation.

A hippocampo-habenular component of the descending column of the fornix has been described on the basis of the examination of normal material (Young, Humphrey and others), and has recently been described also in Marchi experiments by Gerebtzoff. Edinger & Wallenberg, and also Probst, do not agree with this conception and were unable to show the existence of such a component in their Marchi material. Moreover, Cajal, in his normal preparations, concluded that there is no connexion between fibres of the fornix and the stria medullaris. Only in one of our cases (R. 2) in which the fornix was cut successfully did the stria medullaris itself escape involvement by the lesion, and in this case no terminal degeneration was found in the habenular nuclei. The lesions in the other experiments injured the medial superficial fibres of the stria medullaris, and degeneration was traced from the primary lesion

* After interrupting the mammillo-tegmental tract in the rabbit, van Valkenburg observed retrograde degeneration in the dorsal part of the medial mammillary nucleus. The ventro-medial part remained intact, together with the mammillo-thalamic tract. If this evidence is valid, then, at least in the rabbit, impulses received from the fornix would be conveyed directly to the anterior thalamic nucleus and Cajal's concept that fibres of the mammillo-thalamic tract are collaterals of the older mammillo-tegmental tract would not be acceptable.

mainly into the medial habenular nucleus (Text-fig. 2). Thus, although the possibility that in case 2 not all fibres of the fornix were severed cannot be entirely excluded, it seems justifiable to conclude that the existence of a cortico-habenular component of the fornix is at least very doubtful.

It is of interest to note that fibres of the stria medullaris terminate in the medial habenular nucleus by way of boutons terminaux, which, after axonal interruption, swell and become argentophil (Pl. 2, fig. 3). It appears, however, that the synaptic connexions established by the fibres of the fornix system as a whole are made by means of free endings, which by contrast show a drop-like disintegration after axonal interruption (Pl. 1, fig. 1). In cases 1 and 5 the difference in the mode of termination of the two tracts was observed in the corresponding parts of the same section.

Gerebtzoff recently described Marchi degeneration in the pineal body, after sectioning the fornix. In two of our experimental cases in which the stria medullaris was severely injured, fibre degeneration in the pineal stalk was found. In other cases with no significant injury of the stria medullaris the pineal stalk was consistently normal. Thus, our results do not provide evidence for a direct connexion between the hippocampus and the pineal body.

Fibres of the *precommissural fornix* terminate in the lateral and medial septal nuclei and in the nucleus accumbens. That the degeneration seen in these nuclei is the result of interruption of the fornix system proper and is not due to the involvement of the septum by the lesion is demonstrated in case 2, for in this experiment the fornix was cut on the left side and degenerating fibres could be traced into the septum. On the right side, on the other hand, although the lesion passes deeply into the lateral septal nucleus itself, leaving the fornix almost intact, the septal nuclei and also the fibres of the fornix column are almost free of degeneration. Moreover, in case 4 (Text-fig. 6), the lesion is deep and sufficiently far forward to sever those collaterals or direct fibres of the fornix—beautifully illustrated by Cajal (1911, figs. 505 and 511)—which pass out at right angles into the septum. The resulting degeneration in the septal nuclei and the nucleus accumbens was found to be the same as that observed when the fimbria alone has been cut. Apart from terminating fibres, fascicles of fibres from the fornix traverse the septal nuclei to converge into a subcallosal bundle of fine degenerating fibres. In our opinion most of these terminate in the nucleus accumbens, while others appear to join the medial forebrain bundle and pass caudally to an undetermined destination. This may represent the olfactory part of Zuckerkandl's (1888) 'Riechbündel des Ammonshorns', which, according to him, terminates in the olfactory lobe (prepiriform cortex). The preoptic region, the olfactory tubercle and the nucleus of the diagonal band of Broca were normal in all cases.

Descriptions of normal material had suggested a crossing of fornix fibres over the midline anterior to the anterior commissure. Examination of R.2 (right side) and R.5 now make it clear that such a crossing does not exist and that the projection of the precommissural fornix is unilateral.

It is not possible to determine from our material whether different parts of the hippocampus project to different terminal areas. Fox's experiments (1943) have provided some evidence for a rough topographical localization in the hippocampus. He found that in the cat precommissural fornix fibres degenerate after lesions of the

anterior end of the inferior portion of the hippocampus, and that they could be traced to the lateral septal nucleus and also to the dorsal surface of the nucleus accumbens.

The dorsal fornix, as most observers agree, consists of afferent and efferent systems, and the experimental method which we have employed has enabled us to trace many of these fibres to their destination. In the rabbit, the dorsal fornix is composed of two fibre types—a fine fibre component and a group of thicker fibres.

In case 3, the dorsal fornix was selectively cut without injuring any other part of the fornix projection system. It is apparent from the sections of this case that the fine degenerating fibres of the descending dorsal fornix turn into the descending column of the fornix as it curves through the septum (Text-fig. 5) and pass with the column through the hypothalamus and mammillary body to contribute directly to that component of the fornix which projects into the tegmentum. The lateral septal nucleus receives only a few fibres, and the medial septal nucleus and the nucleus accumbens none. In case 2 (right side, Text-fig. 4), this pathway was confirmed. The evidence presented permits the inference that the descending fibres of the dorsal fornix project largely into the tegmentum.

The origin of the descending fibres of the dorsal fornix has been a matter for conjecture. Of the considerable literature written on the subject, Kölliker's (1894) description of the dorsal fornix still appears to be substantially correct. He describes the dorsal fornix as a 'tract of longitudinal fibres which penetrate the corpus callosum from the deepest layer of the cingulate gyrus and also from the superior part of the hippocampus and terminate in the septum pellucidum and in the descending column of the fornix'. He goes on to say that some fibres pass into the septum at the point where fibres of the dorsal fornix join the descending column. Others penetrate the more rostral part of the corpus callosum and enter the septum directly. The latter observation accounts for the small amount of degeneration observed in the septal nuclei in cases 2 (right side) and 5. In normal material fibres have been described passing between the supracallosal striae (Kölliker's deepest layer of the cingulate gyrus) and the dorsal fornix, perforating the corpus callosum, and it has been suggested that they are mainly related to the septal areas. The supracallosal striae arise largely in the indusium griseum, that is, the supracallosal remnant of the hippocampus where Humphrey (1936) found (in the bat) all elements of the hippocampus represented. It would be possible, therefore, to regard the penetrating fibres of the striae Lancisii, and with them the descending fibres of the dorsal fornix, as a remnant of the supracallosal hippocampus-fornix system which rejoins the main column of the fornix in its course through the hypothalamus and, together with some fibres of the descending fornix, passes into the tegmental region.

In all the experiments some of the thick ascending fibres of the dorsal fornix were found to be degenerated posterior to the lesion. These fibres penetrate the corpus callosum, join the deep layers of the cingulum, and apparently curve around the splenium of the corpus callosum to terminate in the hippocampus. However, it cannot be said from our material whether the fibres curving around the splenium are those of the dorsal fornix or are derived from the cingulum, whether the changes observed in the hippocampus are the result of lesions in the ventral or dorsal hippocampal commissures or the dorsal fornix, or whether they are, as in case 5, due to the

immediate injury to the hippocampal formation. At the same time, it must be noted that in this material the medially placed thick fibres associated with the columns of the fornix, which curve up through the septum and enter the dorsal fornix, have always been found to be normal, even when the entire descending column is degenerated. The experimental work of Gerebtzoff (1939) elucidates this matter. By a series of well-placed lesions in the medial telencephalon and the medial frontal and cingulate cortex,* he found that the cingulum could be excluded as a direct afferent pathway to the hippocampal formation, but concluded that the dorsal fornix (colonne horizontale), which arises (according to Gerebtzoff) ventro-medial to the septal nuclei (nucleus of diagonal band of Broca) provides one of the afferent projections to the hippocampus itself.

Hippocampal commissures

The interpretation of the changes in the hippocampal formation itself, which were observed in our experiments, can only be of a very preliminary nature. It is obvious that the greater the lesion in the ventral hippocampal commissure, the more severe are the degenerative changes in the hippocampus (compare cases 1 and 2). The changes are present in the inferior and superior parts of the hippocampus, and all layers, as well as the dentate gyrus, are affected (Pl. 2, fig. 5).† The question whether one area of the hippocampus projects to the same or to different areas of the hippocampus of the opposite side, and the question whether there exists a temporo-ammonic tract which crosses in the dorsal hippocampal commissure (as Cajal propounded) must be left to further study.

It has been established by our experiments that the ventral hippocampal commissure is not a pathway for decussating projection fibres, but is entirely interhippocampal in its connexions. Thus, any influence which the hippocampus of one side may exert on the projection fields of the contralateral fornix must be mediated through the contralateral hippocampus.

SUMMARY

1. Lesions have been placed in various parts of the fornix projection system in the rabbit, and the resulting degeneration of the different components of this system has been followed to their termination by a silver method.

2. It was found that the descending column of the fornix terminates in the basal and intermediate parts of the medial mammillary nucleus. There is no evidence that hypothalamic nuclei which lie in front of the mammillary body, with the exception of the perifornical nucleus, receive any contribution from the fornix. The capsule which surrounds the mammillary body consists partly of fibres from the fornix.

* According to Gerebtzoff, after lesions ventral to and in front of the septum in the rabbit, Marchi degeneration occupying the most medial part of the diagonal band of Broca can be traced through the corpus callosum into the deepest layer of the cingulum and extends into the hippocampus, while fibres of the cingulum remain intact. After lesions resulting in degeneration of the cingulum, the hippocampus was found always to be normal.

† It is interesting to note that the fine fibre and terminal degeneration found in the hippocampal formation in our experiments has never been detected by us either after excision of the olfactory bulb (Le Gros Clark & Meyer, 1947) or after ablation of the prepiriform cortex.

3. The descending column of the fornix and the dorsal fornix contribute to a tract which passes beyond the mammillary body into the mesencephalon.

4. No definite evidence for a hippocampo-habenular or hippocampo-pineal component of the descending column of the fornix could be obtained.

5. Fibres of the precommissural fornix, which are either direct fibres or collaterals of the fimbria, terminate in the lateral and medial septal nuclei and in the nucleus accumbens.

6. Most of the fibres of the descending part of the dorsal fornix pass into the fornix column and continue into the mesencephalon. These fibres may have their origin in the supracallosal remnant of the hippocampus, the indusium griseum. There is evidence that fibres of the ascending component of the dorsal fornix terminate in the hippocampus.

7. Lesions in the ventral hippocampal commissure resulted in fibre and terminal degeneration in all layers of the hippocampus.

8. The entire fornix system is unilateral in its projection, with the exception of some fibres of the descending column of the fornix which cross the midline in the posterior part of the mammillary body.

We wish to express our gratitude to Prof. W. E. Le Gros Clark for his advice and for the facilities offered to one of us (J.M.S.) who was a visitor to his laboratory. We are also indebted to Mr A. C. Allison for help in many ways. Our thanks are due to Mrs Purves for the preparation of the drawings and to Messrs T. Thompson and L. Cooper for technical assistance. The investigation was undertaken while one of us (M.M.) was receiving a grant from the Medical Research Council.

REFERENCES

Allen, W. F. (1944). Degeneration in the dog's mammillary body and Ammonshorn following transection of the fornix. *J. comp. Neurol.* **80**, 283–291.

Cajal, S. Ramon y (1911). *Histologie du système nerveux de l'homme et des vertébrés*, **2**. Paris: A. Maloine.

Clark, W. E. Le Gros (1938). The Hypothalamus. Edinburgh: Oliver and Boyd.

Clark, W. E. Le Gros, & Meyer, M. (1947). The terminal connexions of the olfactory tract in the rabbit. *Brain*, **70**, 304–328.

Edinger, L. & Wallenberg, A. (1902). Untersuchungen ueber den Fornix und das Corpus Mammillare. *Arch. Psychiat. Nervenkr.* **35**, 1–21.

Elliot Smith, G. (1896). The fornix superior. *J. Anat. Physiol.* **31**, 80–94.

Fox, C. A. (1943). The stria terminalis, longitudinal association bundle and precommissural fornix in the cat. *J. comp. Neurol.* **79**, 277–295.

Gerebtzoff, M. A. (1939). Sur quelques voies d'association de l'écorce cérébrale. *J. belg. Neurol. Psychiat.* **39**, 205–221.

Gerebtzoff, M. A. (1941–2). Notes anatomo-expérimentales sur le fornix, la corne d'ammon et leur relation avec diverses structures encéphaliques, notamment epiphysiques. *J. belg. Neurol. Psychiat.* **41/42**, 199–206.

Glees, P. (1946). Terminal degeneration within the central nervous system as studied by a new silver method. *J. Neuropath. exp. Neurol.* **5**, 54–59.

Gudden, B. von (1881). Beitrag zur Kenntnis des Corpus Mammillare und der sogenannten Schenkel des Fornix. *Arch. Psychiat. Nervenkr.* **11**, 428–452.

Humphrey, T. (1936). The telencephalon of the bat. *J. comp. Neurol.* **65**, 603–711.

Johnston, J. B. (1913). The morphology of the septum, hippocampus and pallial commissures in reptiles and in mammals. *J. comp. Neurol.* **23**, 371–478.

KAPPERS, C. U. A., HUBER, G. C. & CROSBY, E. C. (1936). *The comparative Anatomy of the Nervous System of Vertebrates*, **2**. New York: The Macmillan Company.

KÖLLIKER, A. v. (1894). Ueber den Fornix longus von Forel und die Riechstrahlungen im Gehirn des Kaninchens. *Verh. anat. Ges. Jena*, **8**, 45–52.

KÖLLIKER, A. v. (1896). *Handbuch der Gewebelehre des Menschen*, **2**. Leipzig: W. Engelmann.

PROBST, M. (1901). Zur Kenntnis des Faserverlaufes des Temporallappens, des Bulbus Olfactorius, der vorderen Kommissur, und des Fornix nach entsprechenden Extirpations und Durchschneidungsversuchen. *Arch. Anat. Physiol., Lpz*, pp. 338–356.

ROSE, J. (1939–40). The cell structure of the mammillary body in mammals and in man. *J. Anat., Lond.*, **74**, 91–115.

ROSE, M. (1935). Das Zwischenhirn des Kaninchens. *Extr. Mém. Acad. Polon.* Ser. B, pp. 1–108.

VALKENBURG, C. T. VAN (1912). Caudal connexions of the corpus mammillare. *Proc. Acad. Sci. Amst.* **14**, 1118–1121.

YOUNG, M. W. (1936). The telencephalon of the rabbit. *J. comp. Neurol.* **65**, 295–401.

ZUCKERKANDL, E. (1888). Das Riechbündel des Ammonshorns. *Anat. Anz.* **3**, 425–434.

LIST OF ABBREVIATIONS

ant.com.	anterior commissure.	*m.th.*	mammillo-thalamic tract.
b.	basal part of the medial mammillary nucleus.	*n.acc.*	nucleus accumbens.
		n.caud.	nucleus caudatus.
caps.	mammillary capsule.	*n.d.b.b.*	nucleus of the diagonal band of Broca.
c.c.	corpus callosum.	*n.hab.*	nucleus habenulae.
dors.(asc.)f.	dorsal ascending fornix.	*n.hab.l.*	nucleus habenulae lateralis.
dors.(desc.)f.	dorsal descending fornix.	*n.hab.m.*	nucleus habenulae medialis.
d.hip.com.	dorsal hippocampal commissure.	*n.olf.ant.*	nucleus olfactorius anterior.
f.	descending column of the fornix.	*n.perif.*	nucleus perifornicalis.
hab.-ped.tr.	habenula-peduncular tract.	*ocul.n.*	oculomotor nerve.
hip.	hippocampus.	*olf.ped.*	olfactory peduncle.
hyp.	hypothalamus.	*olf.tub.*	olfactory tubercle.
i.	intermediate part of the medial mammillary nucleus.	*op.tr.*	optic tract.
		pre.m.	nucleus premammillaris.
l.	nucleus mammillaris lateralis.	*preop.a.*	preoptic area.
m.	medial part of the medial mammillary nucleus.	*sept.*	septum.
		str.med.	stria medullaris.
mam.b.	mammillary body.	*sub.*	subiculum.
m.ped.	mammillo-peduncular tract.	*v.*	ventricle.
m.teg.	mammillo-tegmental tract.	*v.hip.com.*	ventral hippocampal commissure.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Degeneration of the fine pericellular plexus in the basal part of the medial nucleus of the mammillary body 5 days after section of the fornix. ×1150.

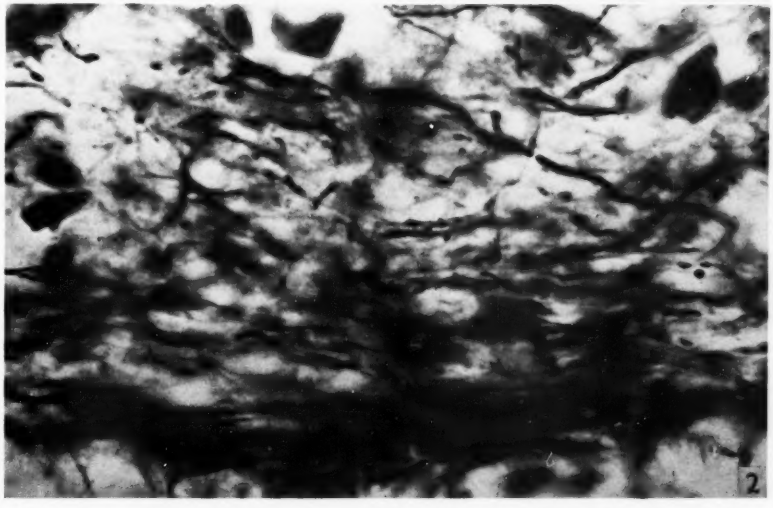
Fig. 2. Degenerating fibres passing into the basal part of the medial nucleus of the mammillary body from the surrounding capsule. ×1150.

PLATE 2

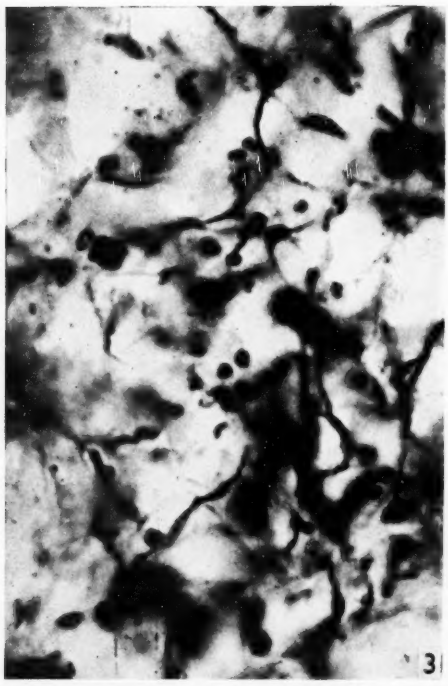
Fig. 3. Degenerating boutons terminaux in the medial habenular nucleus 3 days after incidental injury of the stria medullaris. ×1600.

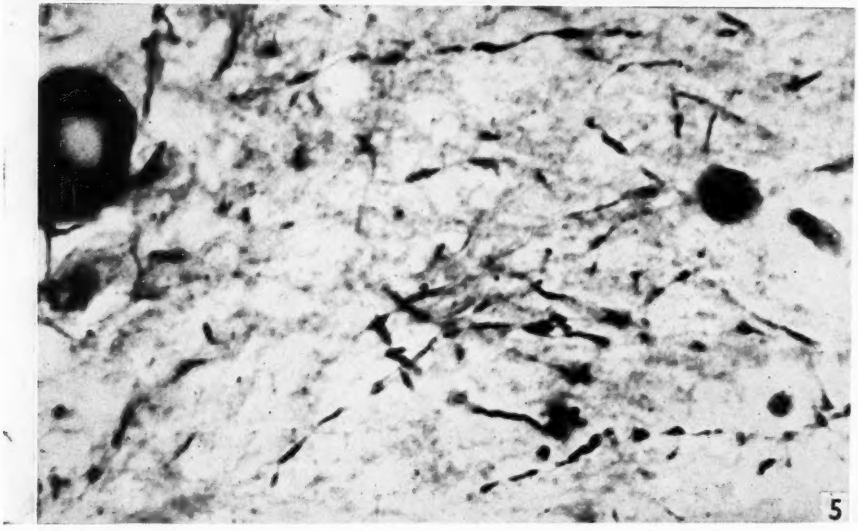
Fig. 4. The normal fibre pattern of the medial habenular nucleus. ×1600.

Fig. 5. Degenerating fibres passing through the molecular layer of the hippocampus towards the dentate gyrus 5 days after an extensive lesion in the hippocampal commissure. ×950.



SPRAGUE AND MEYER—AN EXPERIMENTAL STUDY OF THE FORNIX IN THE RABBIT





SPRAGUE AND MEYER—AN EXPERIMENTAL STUDY OF THE FORNIX IN THE RABBIT

ALKALINE AND ACID PHOSPHATASE DISTRIBUTION IN NORMAL AND REGENERATING TADPOLE TAILS*

By L. C. U. JUNQUEIRA

Department of Histology and Embryology, Faculty of Medicine, University of São Paulo, Brazil

Regenerative processes are of importance in biology in relation to normal and abnormal growth. Needham (1942) gives a full discussion of this problem. Although several aspects of the biochemistry of regeneration have been thoroughly studied (cf. Needham, 1942; Brachet, 1947), the participation of phosphatase in regeneration processes has only recently received attention. In this paper we present the results obtained in studying the acid and alkaline phosphatase distribution in normal and regenerating tadpole tails.

MATERIAL AND METHODS

More than 200 tadpoles of *Bufo marinus* with length between 15 and 30 mm. were used. In order to avoid complicating factors due to metamorphosis, tadpoles whose hind limbs had emerged were discarded. Regenerating tips were observed 2, 5, 10, 15, 20 and 30 days after the tail had been cut. For the demonstration of alkaline phosphatase Gomori's (1939) technique at pH 9·4 was used. Whole tails were fixed in chilled acetone (4° C.) for 24 hr. and washed in distilled water for 20 min. They were incubated for 15–20 min. at 37° C. Controls were incubated in the same mixture without glycerophosphate or with glycerophosphate plus 0·01 M-sodium cyanide.

At the beginning of this study this same technique was tried also on thick sections (20–50 μ) of paraffin-embedded tails, but was discarded because not only did the acetone-fixed fibroblasts appear shrunken but also there was a reduction in the alkaline phosphatase activity; for example, to obtain satisfactory preparations the incubation time of these sections had to be increased from 20 min., used in the whole tails, to 2 hr.

Acid phosphatase was studied using Gomori's technique as described by Glick (1949). The best results were obtained by fixing the tails in 10 % formol at room temperature for 2–4 hr. After repeated washing in distilled water they were incubated at pH 4·5 and at a temperature of 37° C. for 1–3 hr. Controls were incubated with 0·01 M-sodium fluoride.

No attempt was made to study acid phosphatase distribution in sections of paraffin-embedded tails, due to the unreliability of this method with embedded tissues. For a study of this problem we refer to Rabinovitch, Junqueira & Fajer (1949).

Besides these histochemical methods several tadpoles of different ages were fixed in Bouin's, Helly's, Stieve's and Carnoy's fluids, and whole tails or paraffin-embedded sections were stained with haematoxylin-eosin, Heidenhain's ferric haematoxylin or Mallory's trichrome as previously described (Junqueira & Martins, 1947).

* This study was aided by grants from the Rockefeller Foundation and Comissão de Pesquisa Cientifica da Universidade de São Paulo.

These preparations were used as controls for the acetone fixation but none of these methods gave the richness in detail of stained structures as obtained by the phosphatase methods.

RESULTS

A. *Normal tails.* In tails treated by alkaline phosphatase technique the nuclei and cytoplasm of the fibroblasts are intensely stained. The cytoplasm stains diffusely and one can distinctly observe the profuse ramification of its expansions (Pl. 1, figs. 1, 2).

The number and form of the fibroblasts vary with the size (age) of the tadpoles studied and the region of the tail observed. They are more abundant in those regions closer to the central muscular portion of the tail, and scarcer in the peripheral portions. This is due to the fact that the tail is thicker in the middle than at the periphery (Pl. 1, fig. 11). As to the age, tails of younger tadpoles not only have more fibroblasts than those of older ones, but these cells stain more darkly, indicating a stronger alkaline phosphatase activity (Pl. 1, figs. 3, 4). In the tip of the tails the fibroblasts stain more intensely and present their expansion much more ramified, assuming frequently the form of a fan with the base directed medially (Pl. 1, fig. 5). This is particularly evident in the tails of young tadpoles, but is less visible in older ones. The number of these 'fan-like' cells and the intensity of their staining reaction is inversely proportional to the age of the tadpoles observed (Pl. 1, figs. 6, 7).

Besides the fibroblasts, nerve axons give a moderately positive reaction for alkaline phosphatase, while in the Schwann cells only the nuclei stain (Pl. 1, fig. 9).

The nuclei of the epithelial cells give a positive reaction and the cytoplasm a negative one. The epithelial intercellular bridges react positively when sections of paraffin-embedded tails are incubated for 2 or 3 hr. (Pl. 1, fig. 8).

Although this reaction can be observed in whole-tail preparations, due to factors unknown to us, it is better seen in sections.

The cytoplasm and nucleus of the capillary endothelial cells present always a very strong reaction (Pl. 1, fig. 2). In controls incubated without glycerophosphate none of the above-described reactions can be observed. In tails incubated in Gomori's mixture plus 0·01 M-sodium cyanide a strong but not complete inhibition of the alkaline phosphatase activity can be observed (Pl. 1, figs. 10, 11).

It is impossible with the method used to observe the presence or absence of alkaline phosphatase in the chromatophores due to the fact that these cells are already full of dark-staining pigment granules.

There is no possibility of confusion between fibroblasts and chromatophores, for the two types of cell have quite different morphological characters. In addition, it is also possible to observe the position and form of the pigment cells before and after the phosphatase reaction, and so to avoid any mistake.

In tails fixed in formol a diffuse acid phosphatase activity can be observed in nuclei of all the cell types. It is interesting to note that in the nuclei of fibroblasts and epithelial cells we can observe dark-staining corpuscles by the use of both acid and alkaline phosphatase techniques. It is impossible to say, with the techniques employed, whether these corpuscles are nucleoli or chromo-centres. The cytoplasm of the fibroblasts presents a very weak reaction for acid phosphatase, but one can observe a moderate quantity of dark-staining granules in its interior.

B. *Regenerating tails.* Regenerating tails which had been incubated simultaneously with controls show always a stronger alkaline phosphatase activity than normal tails (Pl. 2, figs. 12, 13). Not only is the number of fibroblasts greatly increased in the regenerating tail but these cells are larger, have more ramified processes and show a stronger phosphatase reaction compared with the controls. This fibroblast proliferation attains its maximum degree of staining around the 15th day after operation. It begins about the 4th day and decreases after 1 month. It is interesting to note that the increase of alkaline phosphatase activity is not restricted to the regenerating tip, but also can be observed at the base of the tail.

In the epithelial cells covering the regenerating zone no changes in content and distribution of alkaline phosphatase can be observed, and no variation in the content and distribution of acid phosphatase can be noticed in the cells of normal and regenerating tails (Pl. 2, figs. 14, 15). In controls incubated with 0·01 M-sodium fluoride there is a very strong inhibition of the acid phosphatase activity (Pl. 2, figs. 16, 17).

Whole mounts of tadpole tails were used in this work rather than sections of paraffin-embedded tissues, because in our experience embedding and sectioning distort considerably the appearances of acetone-fixed fibroblasts and partially inactivate the alkaline phosphatase. This inactivation is to be expected, having in view Stafford & Atkinson's work (1948) on the influence of histological procedures on alkaline phosphatase activity.

Our results suggest a participation of alkaline phosphatase in the growth and regeneration of tadpole tails and, without venturing to suggest what may be the exact role of this enzyme in these processes, it is interesting to correlate our work with that of Fell & Danielli (1943), Bourne (1943, 1948), Biesele & Biesele (1944), and Danielli, Fell & Kodicek (1945), who showed an increase of alkaline phosphatase in wound healing. These last authors observed a relation between this enzyme and sites of collagen fibre production. It is of interest that Bradfield (1946) and Jeener (1947) are of the opinion that alkaline phosphatase has a role in the synthesis of oriented protein molecules.

For a thorough review on functions of phosphatases we refer to Moog (1946) and Sols (1949).

SUMMARY

This work has shown that the fibroblasts of the tail of young tadpoles (15 mm.) are larger, more ramified and apparently contain more alkaline phosphatase compared with older animals (30 mm.). In regenerating tails we observe an increase in size, number and enzyme activity of the fibroblasts.

A positive reaction for this enzyme can be observed in the nuclei of all the cells studied, in the axons of nerve fibres, in the capillary endothelial cells and in the intercellular bridges of epithelial cells.

These observations suggest a relation between alkaline phosphatase, growth, and regeneration in tadpole tails.

Acid phosphatase can be detected in the cell nuclei, and in granules contained in the fibroblast cytoplasm. The data obtained do not suggest a participation of acid phosphatase in growth and regeneration of tadpole tails.

The author is grateful to Prof. V. Hamburger for facilities and suggestions during the initial stage of this research.

REFERENCES

BIESELE, J. J. & BIESELE, M. (1944). Alkaline phosphatase in mouse skin under methylcholanthrene treatment. *Cancer Res.* **4**, 751–755.

BOURNE, G. (1943). The distribution of alkaline phosphatase in various tissues. *Quart. J. exp. Physiol.* **32**, 1–19.

BOURNE, G. (1948). Alkaline phosphatase and vitamin C deficiency in regeneration of skull bones. *J. Anat., Lond.*, **82**, 81–87.

BRACHET, J. (1947). *Embryologie Chimique.* Paris: Masson et Cie.

BRADFIELD, J. R. G. (1946). Alkaline phosphatase in invertebrate sites of protein secretion. *Nature, Lond.*, **157**, 876.

DANIELLI, J. F., FELL, H. B. & KODICEK, E. (1945). The enzymes of healing wounds. II The effect of different degrees of vitamin C deficiency on the phosphatase activity in experimental wounds in the guinea pig. *Brit. J. exp. Path.* **26**, 367–376.

FELL, H. B. & DANIELLI, J. F. (1943). The enzymes of healing wounds. I. The distribution of alkaline phosphomonoesterase in experimental wounds and burns in the rat. *Brit. J. exp. Path.* **24**, 196–203.

GLICK, D. (1949). *Technique of Histo- and Cytochemistry.* New York: Interscience Publishers.

GOMORI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23–26.

JEENER, R. (1947). Cytochemical effects of oestradiol. *Nature, Lond.*, **159**, 578.

JUNQUEIRA, L. C. & MARTINS, E. O. (1947). *Atlas de Anatomica Microscopica do Rato.* São Paulo: University Press.

MOOG, F. (1946). The physiological significance of the phosphomonoesterases. *Biol. Rev.* **21**, 41–59.

NEEDHAM, J. (1942). *Biochemistry and Morphogenesis.* Cambridge University Press.

RABINOVITCH, M., JUNQUEIRA, L. C. & FAJER, A. B. (1949). A chemical and histochemical study of the technique for acid phosphatase. *Stain Tech.* **24**, 147–156.

SOLS, A. (1949). Fosfatasas. Revision de la literatura de 1947–1948. *Rev. esp. Fisiol.* **5**, 57–129.

STAFFORD, R. O. & ATKINSON, W. B. (1948). Effect of acetone and alcohol fixation and paraffin embedding on activity of acid and alkaline phosphatases in rat tissues. *Science*, **107**, 279–281.

EXPLANATION OF PLATES

Figs. 8 and 9 are from sections of paraffin-embedded tails; the remainder are from whole mounts.
Figs. 1–7 and 10–13 are from tails stained by Gomori's alkaline phosphatase technique; figs. 14-17 with the method for acid phosphatase.

PLATE 1

Fig. 1. Fibroblast from the tail of a 25 mm. tadpole. Observe strong alkaline phosphate reaction in the cell body and cytoplasmic expansions. 30 min. incubation. ×1500.

Fig. 2. Fibroblast from the tail of a 28 mm. tadpole. Observe stronger nuclear stain as compared with the cytoplasm. In the picture a stained capillary endothelial cell with its nucleus (*C*) and expansions of a pigment cell (*P*). 15 min. incubation. ×1500.

Fig. 3. Tail of a young (15 mm.) tadpole. Note a coarse dark-staining reaction due to the size and amount of fibroblasts present. ×10.

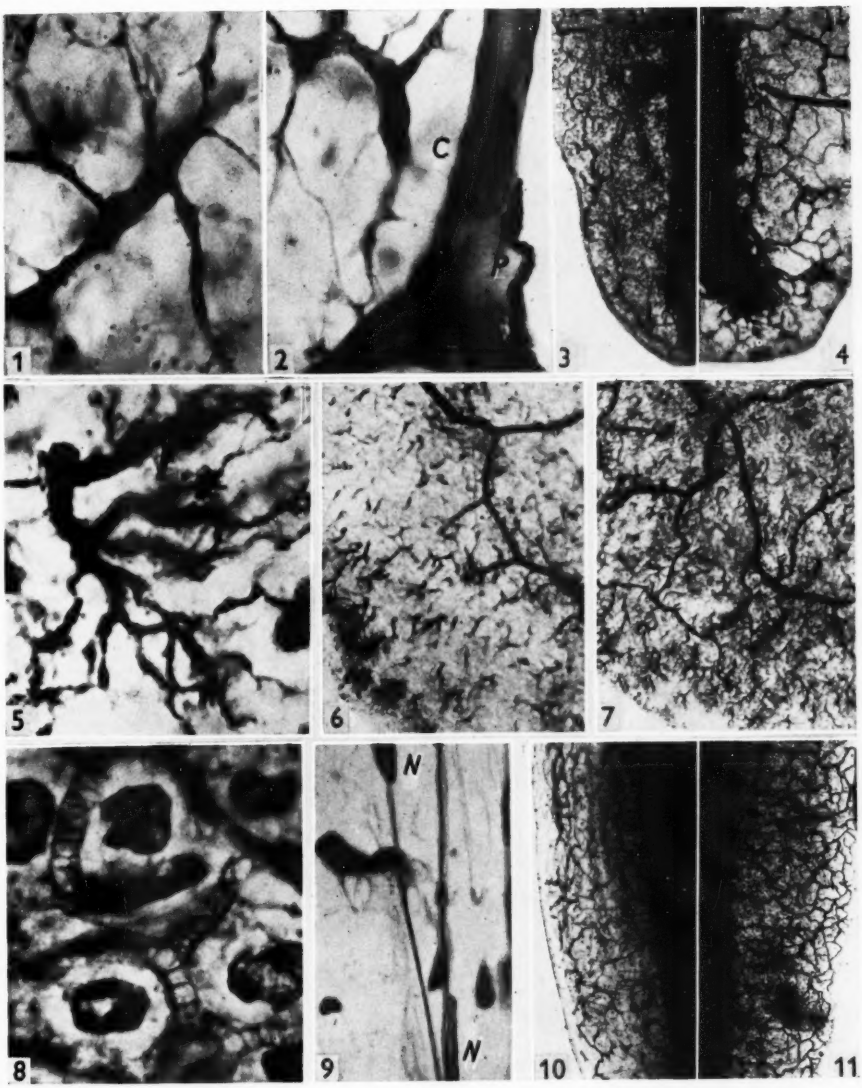
Fig. 4. Tail of a 30 mm. tadpole incubated simultaneously with the tail from fig. 3. Weaker staining due to a decrease in size, number and enzymatic activity of the fibroblasts. ×10.

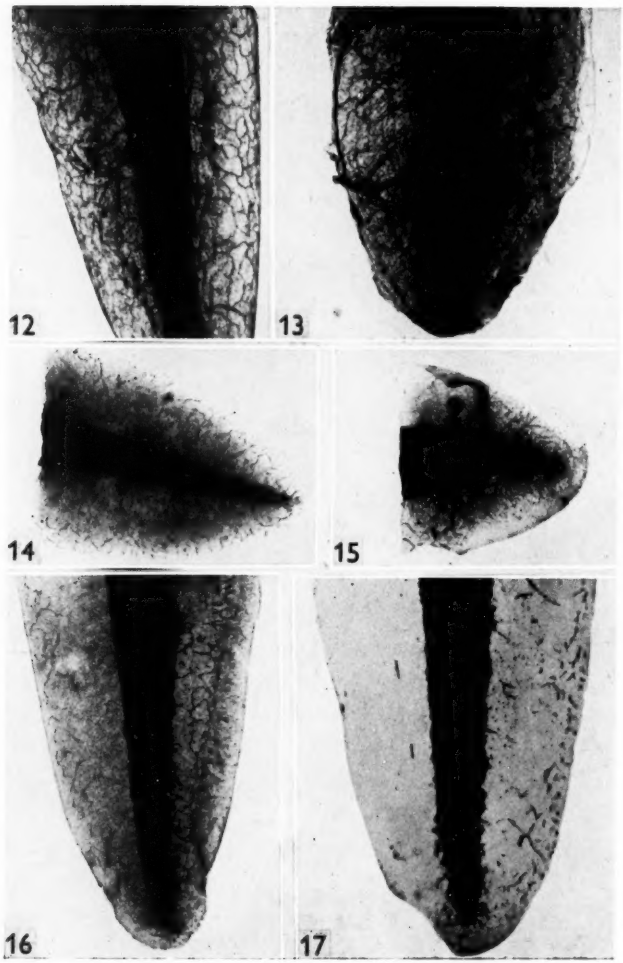
Fig. 5. Fibroblast from the tip of the tail of a 15 mm. tadpole. Observe the strong reaction in the abundant cytoplasmic expansions. Fan-shaped fibroblast. ×1500.

Fig. 6. Tip of the tail of a 27 mm. tadpole. With the exception of a group at the left, the fibroblasts are relatively small and display little cytoplasmic expansion. ×150.

Fig. 7. The same from a 16 mm. tadpole. Fibroblasts are more ramified and abundant. Observe capillaries with intense positive reaction. ×100.

Fig. 8. Epithelial cells with a positive reaction in the nucleus and intercellular bridges. 2 hr. incubation ×1500.


C
P
N
N
1
2
3
4
5
6
7
8
9
10
11



JUNQUEIRA—PHOSPHATASE DISTRIBUTION IN TADPOLE TAILS

Fig. 9. Two nerve fibres. Observe positive alkaline phosphatase reaction in the axon. In the Schwann cells only the nucleus (*N*) stains. Crossing the left nerve fibre is a fibroblast with its expansions deformed due to paraffin embedding. ×1500.

Fig. 10. Control tail incubated with 0·01 M-sodium cyanide. Observe weak reaction as compared with fig. 11. Some blood vessels that simulate a strong positive reaction appear black due to the presence of chromatophores. Isolated spots between the capillaries are pigment cells. ×10.

Fig. 11. Tail of a tadpole of the same age and incubated for the same length of time as in fig. 10. Observe strong reaction and all blood vessels stained. ×10.

PLATE 2

Fig. 12. Tail of a 20 mm. control tadpole. Moderate staining. 20 min. incubation. ×10.

Fig. 13. Regenerating tail of a 20 mm. tadpole 22 days after being cut. Observe intense staining due to increase of alkaline phosphatase activity. Both tails from figs. 12 and 13 were incubated together for the same period of time. ×15.

Fig. 14. Tail of a 25 mm. tadpole incubated for 2 hr. Diffuse staining due to an acid phosphatase reaction in the cell nuclei. ×6.

Fig. 15. Regenerating tail of a 25 mm. tadpole cut 16 days previously. This tail was incubated with its normal control (fig. 14). Observe no difference in the acid phosphatase reaction between normal and regenerating cells. ×6.

Fig. 16. Tail of a 22 mm. tadpole incubated for 2 hr. Observe diffuse staining due to a positive reaction in the cell nuclei. ×10.

Fig. 17. Tail of a 20 mm. tadpole incubated for 2 hr. Incubation mixture plus 0·01 M-sodium fluoride. Inhibition of acid phosphatase activity. The dark-staining dots are isolated chromatophores, and the filaments are portions of capillaries surrounded by these cells. ×10.

THE LOCAL ACTION OF VITAMIN A ON BONE

N. A. BARNICOT, *University College, London*

INTRODUCTION

There is now considerable evidence that vitamin A plays an important part in the metabolism of bone tissue. Deficiency of this vitamin results in abnormalities in the shape of the bones in several species and, as Mellanby (1938) first demonstrated in experiments on puppies, it is the failure of certain bony foramina to enlarge during growth which leads to pressure-degeneration of various nerves. This result was confirmed by Wohlbach & Bessey (1941) in young, but not in adult, rats. In a series of papers (1941, 1944, 1946), Mellanby extended his analysis of the skeletal changes in dogs. He showed that on certain skull bones the position of osteoblasts and osteoclasts became reversed, while in other regions there was a decline of osteoclastic activity. The administration of vitamin A led to the reappearance of osteoclasts in abnormally large numbers in situations where they were normally present (1946). Similar disturbances were detected throughout the skeleton, but the skull and vertebrae were most thoroughly examined. Wohlbach (1947) was able to confirm these results on dogs but gave them a somewhat different interpretation, and Irving (1949), who confined his observations to the incisor alveolar bone of rats, concluded that the basic disorder was excessive osteoblastic activity.

Since these skeletal lesions in vitamin A deficiency can be prevented by very small amounts of the vitamin, this work provides convincing evidence of its importance in normal bone growth, but skeletal disturbances also result if the vitamin is given in amounts greatly exceeding the normal requirement. Following the early demonstration by Takahashi, Nakamiya, Kawakami & Kitasato (1925) of severe toxic symptoms in rats given very large doses of fish liver-oil concentrates (Biosterin), Collazo & Rodriguez (1933) showed that, within 10 days, animals under similar treatment suffered fractures of the limb bones. This was confirmed by Bomskov & Seeman (1933) and Davies & Moore (1934), the latter using a liver-oil distillate containing less impurity than the previously used concentrates. Strauss (1934) made a histological study of bones from hypervitaminotic rats and found that the cortex of the long bones, and also the skull bones were thinned, and the occurrence of fractures was again reported by Hoff & Jeddeloh (1934–5), Weslaw, Wronski, Wroblewski & Wroblewski (1938) and Vedder & Rosenberg (1938). In 1945, Moore & Wang established that the fractures were actually due to vitamin A itself by producing them in young rats which were given 25,000–50,000 i.u. daily as a solution of crystalline vitamin A acetate. Irving (1949) reported that in rats given similar doses, the incisor alveolar bone showed a decrease of osteoblastic activity while osteoclasts remained in normal numbers. There is little published information about the detailed bone histology in hypervitaminosis A, but a summary by Wohlbach (1947) of his experiments on several species, shows that with sufficiently high doses osteoclastic resorption of the cortex of the long bones occurs, particularly in regions of active remodelling, and that with a somewhat lower and more prolonged dosage the shaft

diameters become reduced in relation to their length. The most recent contribution is that of Rodahl (1949), who noted fractures and the same curious narrowing of the long-bone shafts in young rats given either polar bear liver-oil or whale liver-oil concentrates, both of which contain high concentrations of vitamin A. It appears from the literature that in vitamin A deficiency there is a disturbance of the balance of bone deposition and resorption leading to excessive thickness of bone in certain regions, while in overdosage the situation is reversed and the skeleton is weakened. There is at present no evidence to show whether the vitamin exercises its influence on bone by a direct action on osteogenic cells, or whether a more complicated chain of metabolic disturbances is involved. The grafting technique used by the writer (1948*a*) to investigate the local action of the parathyroid on bone seemed well suited to a further analysis of the effects of vitamin A when present in excess. Some preliminary results have already been published (1948*b*), but since then further experiments have been performed, and the full data are reported in detail in the present paper.

MATERIAL AND METHODS

The grafting technique has already been described by the writer (1948*a*), and only a few details need be added. It may, however, be useful to reiterate the logic of the method. The tissue or compound under examination is attached to a small piece of parietal bone cut from a 10-day-old mouse and the two are then inserted into the cerebral hemisphere of a litter-mate and left for 2 weeks. It is argued that if the substance is capable of exerting a purely local action on bone this will be shown by changes in the immediate vicinity of its attachment. If such a change occurs, it is not likely to depend on the stimulation of some tissue remote from the graft, to which the implanted substance would have to be carried in the blood-stream, since in that case effects throughout the skeleton of the host would be expected. It is conceivable, however, that the liberation of a highly active compound from the graft site might result in a systemic effect which would in turn modify the response of the graft bone to the local action of the compound, including, for example, a non-specific action such as the mechanical pressure of the implanted material. If this were so one might expect to observe some effect on the host animal also, but it could still be argued that the systemic change might be sufficient to modify the response of the graft but not to produce any obvious change in the host. While such possibilities have not been rigorously excluded, they do not seem to be very likely.

The material used for what is conveniently referred to as the chemical implant was crystalline vitamin A_1 acetate, which was kindly provided by Sir Edward Mellanby. The ester was kept sealed in carbon dioxide in the refrigerator, and should be fairly stable under these conditions. For many of the experiments a single small chip was broken off and attached to the centre of the endocranial aspect of the piece of parietal, using solutions of human fibrinogen and thrombin with penicillin added. The graft was then thrust vertically into the cerebral hemisphere of the host through a small slit made in the skull roof. In some experiments, which were designed for a more exact comparison of the local actions of vitamin A and calciferol, the vitamin A was finely powdered, and the powder was then cemented into small pellets, of suitable size for grafting, by means of fibrin clot. In some cases either oestradiol or cholesterol was mixed with the powdered vitamin A to see whether the former sub-

stance modified the bone response; since no difference from the effects of vitamin A alone could be detected, these specimens are included in the present description.

The grafts were finally examined either as dry specimens after maceration in 2% KOH, or by histological sectioning. In many cases they were stained supravitally with neutral-red in the manner previously described by the writer (1947) and the distribution of osteoblasts and osteoclasts was recorded by drawing the preparation on millimetre graph paper, using an ocular graticule and a 2 in. objective. The position of osteoclasts could be fairly accurately recorded in this way, but the osteoblasts were too numerous to be individually marked, and the drawings merely recorded the size and shape of the scattered areas occupied by these cells. In this connexion it should be noted that it was necessary to remove a layer of connective tissue from the graft surface either before or after staining, in order that the cells might be clearly visible, and some loss may have occurred in this process; as far as one could tell, however, from dissection of stained specimens under the microscope such a loss was slight, and the curiously patchy distribution of osteoblasts is unlikely to be an artefact. A staining period of 30–45 min. in a $\frac{1}{10,000}$ solution of neutral-red in 0·9% saline was generally sufficient to give strong coloration. If supravitally stained specimens were fixed in 2% mercuric chloride to preserve the stain, subsequent maceration was often not successful and resulted in some disintegration and distortion of the bone.

Histological material was prepared by removing the graft in a large block of brain tissue together with the overlying skull and fixing and decalcifying simultaneously in Zenker + 3% of glacial acetic-acid. The material was double-embedded in celloidin and paraffin and after cutting serially at 8μ was stained with Ehrlich's haematoxylin and orange G-erythrosin. The sections were in a plane parallel to the sagittal plane of the skull and therefore cut the graft bone at right angles to its surface and showed the full length from the skull wound to the deepest edge.

Animals of two inbred strains were used, C 57 black and Glaxo Champagne, and no major difference in the response of the two stocks was noticed.

Macerated and supravitally stained material

The macerated material consists of thirty-four grafts, and their distribution between different types of experiment, together with a classification of the effects on the bone is set out below (Table 1). The table gives the type and duration of the

Table 1

Type and duration of graft	Perforated	Thin area; not perforated	Resorption slight or absent	Total
Vit. A single fragment (14 days)	C 4 A 2	C 2 A 2	A 1	11
Vit. A single fragment (7 days)	A 1	A 2	A 1	4
Vit. A powdered (14 days)	C 2 A 2	A 2	A 1	7
Vit. A + cholesterol (14 days)	C 2	C 3	C 1	6
Vit. A + oestradiol (14 days)	C 5 (3♀–2♂)	C 1 (♀)	— —	6 —
Total	18	12	4	34

graft, and the letters A and C indicate Champagne and C 57 animals respectively. It will be realized that this division into categories is to some extent arbitrary, for the dividing line was not always sharp, and it is probable that a more extensive material could be arranged in a continuous series. The results are on the whole striking and consistent. More than half the bone grafts showed a definite perforation situated in the central region, and in many cases dissection revealed the vitamin residue somewhere within this hole. The perforations were generally more or less circular in outline with a smooth sharp margin, which was often formed by a band of thin bone demarcated by a step from the thicker bone nearer the graft periphery. Neutral-red revealed osteoclasts on some part of this margin (Pl. 1, fig. 8; Text-fig. 5). The

1

Text-figs. 1–5. Scale drawings of supravitally stained specimens showing the distribution of osteoclasts on the surface nearest to the vitamin implant. × 29. Photographs of the bones from which these figures were drawn are included in Pl. 1, so that the correspondence of bone structure and cell distribution can be studied.

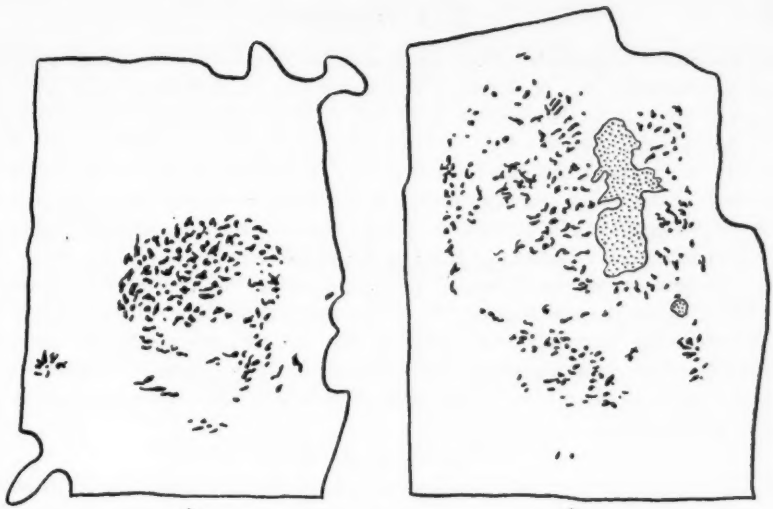
Text-fig. 1. Bone implanted with single fragment of vitamin A, and removed after 7 days. The diagram also shows areas of osteoblasts, indicated by a fine stipple, and canals in the bone. There are several scattered groups of osteoclasts in the central region. Compare Pl. 1, fig. 9, which shows the macerated specimen.

majority of grafts showed a varying degree of thickening along the edge which lay in the insertion wound, but this is a feature which usually occurs in all types of graft, and is independent of the chemical implant. Although a single perforation was more usual, some specimens had one or more smaller holes lying close to the main one, and these may have been due to small particles of vitamin A adherent to the main implant, which became displaced. In some specimens (Pl. 1, fig. 6) there was a tongue of thin bone projecting into the perforation from some part of its margin, and the whole endocranial surface of such a projection might be covered with osteoclasts. The second group of specimens consisted of bones which were not actually perforated but showed a roughly circular area of thinner bone near the central region (Pl. 1,

figs. 4, 5). Such areas were slightly sunk below the surface of the surrounding bone, and often quite sharply demarcated from it by a ledge. The bone itself was sometimes homogeneous and translucent (Pl. 1, fig. 4) and sometimes more opaque and perforated by numerous evenly dispersed foramina giving it a sieve-like appearance (Pl. 1, figs. 5, 7). The thin area did not generally project much on the convex surface. These thin areas were clearly sites of resorption and incipient perforation, since supravital staining revealed numerous osteoclasts covering the whole or part of their surface nearest the vitamin (Text-figs. 2–4). It will be noticed in Text-fig. 3 and Pl. 1, fig. 5, which are of the same bone, that there is a second rather thicker area of resorption within which the thinnest area lies, and that osteoclasts are arranged along its borders. Pl. 1, fig. 7, and Text-fig. 4 show a specimen in which a large area of sieve-like bone is perforated in the centre. It is clear that osteoclastic resorption in these regularly shaped thin areas would ultimately lead to the kind of large perforations already described, and that if perforation occurred unevenly or at more than one position an intermediate stage with a projecting tongue of bone would result. The peripheral regions of the endocranial surface of the grafts showed the same kind of irregular thickening which one finds in grafts implanted with inactive substances or with no chemical implant, and this phenomenon will be mentioned again later. Osteoblasts were observed in the peripheral regions as occasional clusters of irregular shape. The origin of the thin bone will also be discussed again when dealing with the histological material, but it may be remarked here that its structure is distinguishable from the thin bone seen in ungrafted parietals (Pl. 1, fig. 1) and it is not likely to be simply the unchanged bone of the original implant. This is particularly the case with the sieve-like bone, which is, however, not absolutely distinct from the homogeneous type, since transitional forms occur. The regular shape and arrangement of the foramina and the occasional occurrence of fine surface grooves connected with them (Pl. 1, fig. 5) suggest that they carried small vessels. There is a further piece of evidence bearing on the origin of this thin bone, provided by certain grafts which were macerated after mercuric chloride fixation. In these a certain separation of bone layers occurred which enabled one to see that incompletely resorbed thin bone at the margin of a perforation was often continuous with a layer of bone covering the convex surface of the graft. It seems very probable that the layers of bone which are caused to separate by this maceration treatment are in fact newly formed layers which are comparatively loosely attached to the older bone, on the surface of which they were laid down following a pause in osteogenic activity occasioned by the grafting procedure. This suggests, then, that the perforation may involve not only the bone of the original graft but also a newly formed layer.

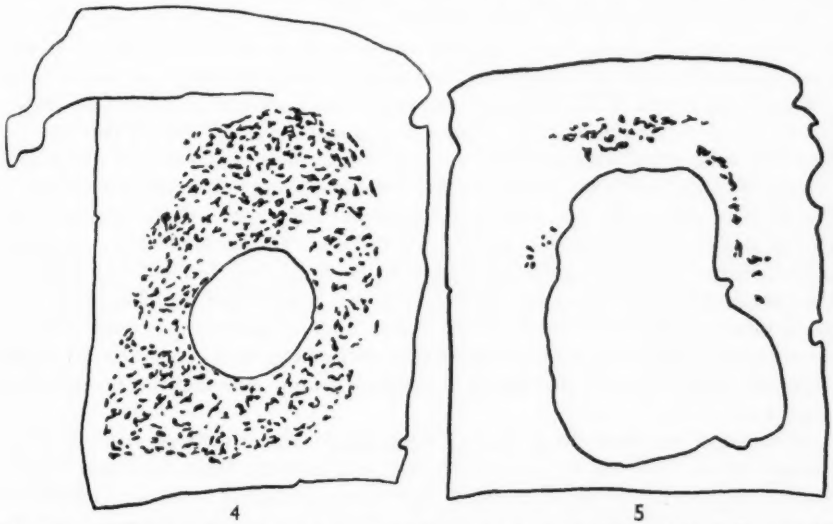
The osteoclasts which are found in resorption areas of vitamin A grafts are in no way peculiar and show a variation of form and staining intensity which could easily be matched in the growing parietals of normal mice as described by the writer (1947).

Six grafts were removed 7 days after the operation and four of them were examined by maceration. They showed relatively slight evidence of resorption; the most advanced stage was one with a small irregular area of thin bone interrupted by several ragged perforations, while in another there was a small, circular area of thinning with ill-defined margins, and bearing a number of osteoclasts. At the other extreme was a specimen in which resorption was only apparent as a minute irregular pit with



Text-fig. 2. Bone implanted with powdered vitamin A + cholesterol; removed after 14 days. Compare the bone structure shown in Pl. 1, fig. 4. Osteoclasts are clustered on a circumscribed area of thin bone. There are also some osteoclasts on the thicker bone below this area.

Text-fig. 3. Bone implanted with powdered vitamin A + cholesterol; removed after 14 days. Compare macerated specimen shown in Pl. 1, fig. 5; osteoclasts scattered over a circumscribed area of sieve-like bone, where the partially dissected remains of the vitamin are also situated (stippled area). Osteoclasts also occur near the margins of a somewhat thicker area of bone which surrounds the first area.



Text-fig. 4. Bone implanted with powdered vitamin A + oestradiol; removed after 14 days. Compare the macerated bone shown in Pl. 1, fig. 7. Osteoclasts are numerous and restricted to a large area of sieve-like bone; the centre of this area is perforated.

Text-fig. 5. Bone implanted with powdered vitamin A + cholesterol. Compare the macerated specimen shown in Pl. 1, fig. 8. There is a large central perforation with osteoclasts located on part of the margin where thin and thick bone adjoin.

a few associated osteoclasts. Pl. 1, fig. 9, and Text-fig. 1 show the fourth specimen in which resorption can be detected as a number of small erosions near which lie clusters of osteoclasts. It is evident from this limited material that the greater part of the resorption in vitamin A grafts must occur during the second week, and this is probably due to the establishment of an adequate vascular supply by the end of the first week, as the writer also suggested in discussing parathyroid grafts (1948*a*) and intracerebral grafts of ribs (1941). It appears from these specimens that resorption may start in several isolated foci close to the vitamin implant and that these then spread to form an extensive area of thinning; during this process the number of osteoclasts increases considerably. It is also worth remarking with regard to the entire macerated material, that, as far as one can tell, the results are the same whether the vitamin is implanted as a single fragment or as a pellet of powder; nor does the inclusion of some 50 % of oestradiol in such a pellet appear to modify the resulting resorption.

Histological material

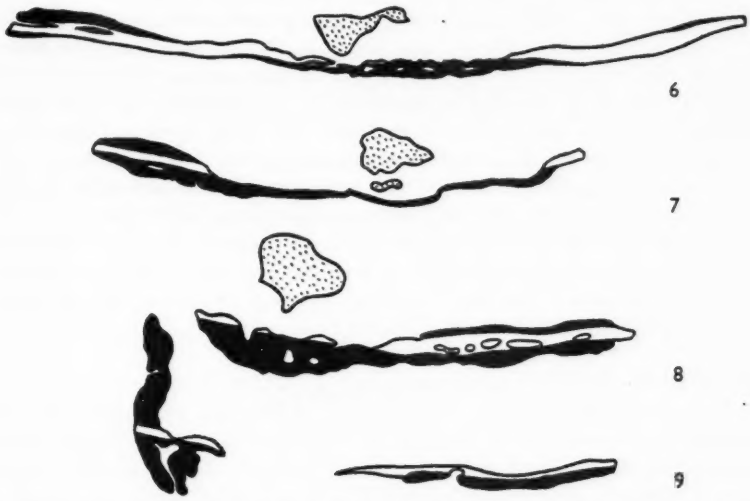
The material consists of eight specimens, in all of which a single chip of vitamin A was used for implantation. Six of the grafts were performed on C 57 animals which were killed after 14 days, and two were performed on Champagne animals, which were killed after 7 days.

Fourteen-day grafts

The vitamin residue was identified in all six specimens, but in two it had become displaced towards the skull wound. Resorption in the vicinity of the chemical implant is illustrated in the two specimens reproduced in Pl. 2, figs. 11, 12. In both of these the vitamin residue, represented by a space, lies close to the bone surface near the centre of the graft, and the bone for a considerable distance on one or both sides is very thin and contrasts with the thicker bone at the two extreme ends. In the specimen shown in Pl. 2, fig. 12, osteoclasts can be found at intervals over the whole extent of this region of thin bone but only on the surface nearest the vitamin. The thin bone itself with its rather homogeneous pale-staining matrix and living osteocytes, is distinctly reminiscent of the new bone found in the resorption areas of parathyroid grafts, but it is more continuous and has a more regular contour. Its structure is shown under higher power in Pl. 2, fig. 14. On the convex, or ectocranial aspect, there are quadrangular osteoblasts of medium size. Immediately opposite the vitamin the bone layer was actually perforated in a few sections. The thick bone has a more complex, laminated structure and contains some empty lacunae in its deeper parts; while it is very probable that part of the thickness is due to newly deposited bone it is not possible to distinguish clearly new and old bone in this specimen.

In the other specimen (Pl. 2, fig. 11), the general structure of the graft is similar; the area of thin bone has become slightly detached from the brain tissue and there is some extravasation of blood, probably due to injury during removal. Osteoclasts appear to be restricted mainly to the region where the thin central bone meets the thicker bone at the edges; the endocranial aspect of the thin bone contrasts, however, with the opposite surface in showing flattened spindle cells and very few large osteoblasts. In the thicker peripheral regions a layer of bone of fairly uniform thick-

ness can be seen lying embedded between two layers which have apparently been deposited on its surface. The condition appears to be essentially similar to that previously described in parathyroid and other tissue grafts, in which the original graft bone, which is often darker staining and may have empty lacunae, is demarcated by cement lines from newer bone laid down on both its surfaces. These



Text-figs. 6–9. Projection drawings of histological sections of grafts. Bone of original graft white, new bone black. Vitamin residue stippled. × 57.

Text-fig. 6. Graft of a single fragment of vitamin A removed after 7 days. Adjacent to the vitamin the original bone is perforated and replaced by spongy new bone. There is also some thickening by new bone deposition at the extreme edges. Compare photomicrographs of this specimen (Pl. 1, fig. 10 and Pl. 2, fig. 13).

Text-fig. 7. Graft of a single fragment of vitamin A removed after 14 days; the central area is composed of new bone and the original bone is found only at the edges. Compare photomicrograph of this specimen (Pl. 2, fig. 11).

Text-fig. 8. Graft of a single fragment of vitamin A removed after 14 days. The vitamin is somewhat displaced towards the skull wound. Opposite it the original bone graft has been perforated but the holes are sealed by a heavy deposit of new bone. The exact distribution of new and old bone on the right-hand side of the figure is difficult to make out in the specimen and is somewhat conjectural in the diagram.

Text-fig. 9. Same specimen as Text-fig. 8 at a different level in the series and showing actual perforation. Note extensive plate of new bone at the extreme left side; this lay in the scar tissue of the insertion hole.

relationships are shown more clearly in Text-fig. 7 which is made from a projection drawing of the same specimen. It will be noticed that the layer which is taken to represent the original graft is sharply discontinuous and is in fact perforated, but that the hole is bridged by the thin bone continuous with the new bone layer on the convex aspect. In the two specimens in which the vitamin was displaced towards the surface edge of the graft there is a considerable perforation in the adjacent region,

and the gap is bridged by fibroblasts. In one of these specimens the perforation, which is shown in Text-fig. 9, lies to one side of the chemical implant, opposite which the bone is continuous, but lined with osteoclasts. It can be clearly seen that much of the original graft is here perforated and that a thick layer of new bone which is also being resorbed, closes the gaps (Text-fig. 8). At the deep end of the graft new and old bone are not easily distinguishable; this is probably due to the fact that the original graft bone may have had a somewhat spongy structure owing to the inclusion of part of the lateral area of the parietal bone. In the fifth specimen the bone was continuous but, somewhat to one side of the vitamin residue, there was a zone of osteoclastic resorption forming a concavity; it is probable, that the bone in this region was also largely a new formation. The sixth specimen showed a few osteoclasts near the vitamin, but otherwise no evidence of resorption, and the reasons for this negative result are not apparent. If one attempts to link up these histological findings with the observations on macerated material, it seems probable that the two specimens with extensive areas of thinning correspond to those macerated bones showing areas of homogeneous thin bone, but nothing obviously corresponding to the sieve-like bone has been observed. One of the 7-day specimens throws some light on this matter, however.

Seven-day grafts

The specimen is illustrated in Pl. 1, fig. 10 and Pl. 2, fig. 13, and the structure is illustrated diagrammatically in Text-fig. 6. Opposite the angular vitamin fragment, and extending beyond it on one side, there is a length of bone which is spongy in structure, slightly paler staining, and contains large rounded osteocytes. This layer is continuous with a new layer of bone on the convex surface of the original graft bone, which can be distinguished at either side, and in which the lacunae are mainly empty. Osteoclasts are to be seen on the surface of the bone nearest the chemical implant and extending some distance on either side of it. In this specimen perforation and closure are therefore quite clear, and the new bone has a structure which may correspond to the sieve-like bone of macerated material. The series of the other 7-day specimen was rather incomplete; the graft was uniform in thickness with a thin layer of new bone and active osteoblasts on its ectocranial surface but only a few osteoclasts near the vitamin as evidence of a local response.

Chemical changes in the vitamin implant

Although the acetate is described as a comparatively stable ester of vitamin A when kept in air at 0° C. (Baxter & Robeson, 1942), it might well undergo oxidative destruction when implanted in the tissues at much higher temperatures. In some of the histological sections the vitamin residue had an angular form, but in others it was more rounded, and this, together with the proximity of numerous macrophages with vacuolated cytoplasm and occasional polymorphs suggested that the vitamin might be undergoing some decomposition or change of physical state. It was also noticed that when the grafts were removed the vitamin residue was opaque and that if it was mounted under a cover-slip it easily disintegrated into a fine granular material. Sometimes a core of translucent substance, like the originally implanted fragment, remained at the centre of such a granular mass. Dr S. P. Datta and Mr B. G. Overell

kindly examined a number of these residues by the method of paper chromatography for vitamin A and its derivatives which Datta, Overell & Stack-Dunne (1949) have described. The implants in these cases were of powdered vitamin made into pellets with fibrin so that the fine state of division may have favoured decomposition. While chromatographs of the material from which the implanted pellets had been made gave a strong spot for A-ester together with a trace of A-alcohol and of some unidentified derivative, no trace of vitamin A or its derivatives could be observed by this procedure in residues from 14-day grafts. Direct colorimetric estimation in solution using the Carr-Price method was therefore undertaken on pellets after 14 days in graft and also on some of comparable size which had not been grafted. The results are shown below:

Fresh pellets (i.u.)	Grafted pellets (i.u.)
(1) 19·0	(1), (2), (3) Pooled 2·0
(2) 26·0	
(3) 16·5	
(4) 22·5	(4) 1·0
	(5) 0·5

It is clear that there is very little Carr-Price chromagen left in the grafted pellets, the amount present being close to the limits of sensitivity of the method.

Grafts of β-carotene

Before discussing the results as a whole, seven macerated specimens of 14-day grafts of β-carotene must be described briefly. Since β-carotene is a substance chemically allied to vitamin A, and, indeed, is known to act as a precursor or provitamin from which vitamin A can be formed in the body, it was of some interest to examine its local action on bone. The small crystals were cemented together with fibrin for implantation and the grafts were performed on C 57 animals. On removal, the clumps of deep red crystals were easily detected lying near the centre of the bones and their sharp-edged form suggested that they had not dissolved appreciably. A few osteoclasts and scattered irregular patches of osteoblasts were detected with neutral-red, but their distribution had no obvious relation to the site of the carotene implants. The macerated bones (Pl. 1, fig. 3) showed a variable amount of irregular thickening. Similar bone structure and cell distributions are found in grafts carrying no chemical implant but simply treated by clotting fibrin on the endocranial surface (Pl. 1, fig. 2). Five grafts of this kind were performed and left for 14 days, and three for 26 days. In the latter the contrast between thick and thin areas was more pronounced. The sparsity of osteoclasts on β-carotene grafts and on those without chemical implant suggests that the relatively thin areas in such grafts are not a result of resorption but are, in the main, areas of the original graft unthickened by secondary deposition. By comparing plots of the supravitally stained cells with the macerated material it can also be seen that osteoblasts generally occur at the junctions between thick and thin areas, suggesting that in the thickest parts deposition has slowed down or ceased. One can safely conclude that β-carotene produced no local resorption or other significant change in the bone. It may be that too little dissolved to give any reaction, or it may be that β-carotene itself is in fact inactive in this respect and that insufficient conversion to vitamin A occurs in the tissues, under these conditions, to produce local resorption.

DISCUSSION

The work presented here makes it clear that vitamin A acetate is capable of producing active local bone resorption if it is brought into close contact with the surface of young bone. This result seems, in general, to be consistent with reports of experiments on the whole animal, which show that bone overgrowth is the major result of vitamin A deficiency and that resorption and weakening of the skeleton occur if the vitamin is given in excess. There is no good reason to think that in the graft experiments resorption results from other than a local action since the host animals showed none of the signs of general hypervitaminosis such as a failure to grow, unkempt hair, or inactivity; nor did there appear to be a generalized skeletal resorption in the host and the resorption of the graft was confined to the neighbourhood of the vitamin. It can in fact be calculated from the analyses of the vitamin content of the implants, that even if the whole implant were given systemically as a single dose and were completely absorbed it would still be only about one-tenth of the dose required to produce hypervitaminosis, assuming the body weight/dose relationship to be of the same order as it is in young rats. It is still not possible, however, to determine the exact locus of action of the vitamin in these experiments since the graft is a fairly complex structure which includes other tissue elements, besides the osteogenic cells. There is some evidence, derived from the writer's observations on parathyroid grafts (1948*a*) that, during the first few days after grafting, the osteoblasts on the surface of a graft become a disorganized layer of spindle-cells and that any osteoclasts which may have been present disappear. One finds very few osteoclasts on grafts of bone without chemical implants, or on those implanted with certain inactive substances, and it has been demonstrated here that in grafts removed after 7 days they are restricted to the region of the vitamin, and that after 14 days their number has increased considerably. The vitamin therefore seems to stimulate the differentiation and multiplication of these cells, and perhaps also their functional activity. Whether it does so by acting directly on them or on their precursors, which seems the most likely mechanism, or by some indirect means such as the control of the local vascular supply, one cannot say. Certainly resorption does not seem to start until a vascular supply is established, but this may simply mean that the response of the cells or the ability of the vitamin to reach them is limited by this factor. It is also important to notice that osteoblastic activity in the resorption areas is by no means suppressed, although on the actual surface where osteoclasts are numerous, active osteoblasts are seldom found. Irving's (1949) hypothesis, that the vitamin acts by suppressing osteoblastic activity, while leaving osteoclastic resorption unchanged but uncompensated, cannot apply here, since osteoclasts appear to be formed *de novo* in the vicinity of the implant, and to resorb the bone in its immediate neighbourhood, while simultaneous osteoblastic activity at the periphery and on the opposite surface of the bone may be considerable.

It would be helpful to know the actual local dosage of vitamin A in the graft experiments, but for various reasons no useful estimate can be made. There is not enough known about the solubility of the vitamin when present in the solid form, about the properties of the surrounding solvent medium, nor about the rate of the removal of the dissolved material from the site of action either by decomposition or by replacement of the solvent. In addition to all this the evidence that the ester is

progressively destroyed raises the further question whether it is in fact the ester or some derivative which stimulates resorption. According to Clausen, McCoord, Baum, Steadman, Rydeen & Breese (1940) the normal blood vitamin A in man is in the form of the alcohol, and Glover, Goodwin & Morton (1947) have presented evidence that the blood vitamin level in rats is more closely correlated with the level of A alcohol in the liver than with the level of the esterified vitamin in that organ, which suggests that the latter is a storage compound. Although the blood vitamin A may be very much increased in hypervitaminotic rats, as Walker, Eylenburg & Moore (1947) and other workers have shown, it is not known whether this rise is due to the ester or to some other form. The investigation of other vitamin A compounds by the present technique would be of some interest.

Turning to the facts disclosed by Mellanby's work, it is apparent that the situation in A deficiency is not simple. Osteoclasts are by no means absent, but they occur in unusual situations, and the precise character of the defects is not the same in different regions of the skeleton. In experiments on the whole animal either vitamin deficiency or excess is likely to lead to changes in tissues other than the skeleton, and even if the effects on the bones are to some extent direct ones, they are liable to occur in an environment which has been rendered abnormal in various other respects. Since, according to the commonly accepted view, osteoclasts and osteoblasts perform complementary functions, which in normal growth are somehow spatially co-ordinated, it would not be surprising if vitamin A and also other essential substances affected both cell types but in an opposite sense. The precise result of deprivation or excess of the substance in any particular case might then be expected to depend on such factors as its local concentration at the bone surface, and the degree of activity of the two cell types at that particular region of the skeleton and at that particular developmental period, and this would at the same time be influenced by various other factors on which the metabolic activity of bone tissue also depends. It is possible that some of Mellanby's observations might be unified by postulating that deprivation of vitamin A affects the response of the bone tissue to mechanical pressure, so that in some areas resorption no longer results from a given degree of pressure exerted by adjacent organs, while in others the response is reversed and deposition of bone occurs. The continual rise of pressure due to this defect might explain why such an abnormally great outburst of osteoclastic activity occurs when the vitamin is again supplied. It may be pointed out, in conclusion, that even if vitamin A deficiency or excess leads to changes in both osteoblastic and osteoclastic activity by a predominantly local action, various possible mechanisms might be suggested between which one cannot decide; it might act on each of the two cell types, or on only one of them, the correlated response of the other being mediated by some essentially independent mechanism, or again it might act mainly on some common precursor cell causing differentiation in one or other direction. Our knowledge of the mode of origin and fate of osteoclasts is not at present sufficient, however, to provide a secure basis for further speculations.

SUMMARY

1. Fragments of crystalline vitamin A acetate were attached to small pieces of parietal bone cut from 10-day-old mice, and the combination inserted into the cerebral hemisphere of litter-mates.

2. The grafts were removed after 7 or 14 days and were examined either by supravital staining with neutral-red followed by maceration or as histological sections.

3. Well-marked resorption, accompanied by numerous osteoclasts, and often leading to perforation of the bone was apparent after 14 days.

4. The significance of the results is discussed in relation to existing knowledge of the skeletal effects of vitamin A deficiency and of overdosage with this vitamin.

My thanks are due to Miss I. M. Slowgrove for technical assistance with the histology and the photography of the macerated material, and to Mr F. J. Pittock and Mr J. Armstrong for the photomicrographs. The work was accomplished with the help of a grant from the Dixon Fund of the University of London.

REFERENCES

Barnicot, N. A. (1941). Studies on the factors involved in bone absorption. I. The effect of subcutaneous transplantation of bones of the grey-lethal house mouse into normal hosts and of normal bones into grey-lethal hosts. *Amer. J. Anat.* **68**, 497–531.

Barnicot, N. A. (1947). The supravital staining of osteoclasts with neutral-red; their distribution on the parietal bone of normal growing mice and a comparison with the mutants grey-lethal and hydrocephalus-3. *Proc. roy. Soc.* B, **134**, 467–485.

Barnicot, N. A. (1948*a*). The local action of the parathyroid and other tissues on bone in intracerebral grafts. *J. Anat., Lond.*, **82**, 233–248.

Barnicot, N. A. (1948*b*). Local action of calciferol and vitamin A on bone. *Nature, Lond.*, **162**, 848.

Baxter, J. G. & Robeson, C. D. (1942). Crystalline aliphatic esters of vitamin A; crystalline vitamin A. *J. Amer. chem. Soc.* **64**, 2407–2411.

Bomskov, C. & Seeman, G. (1933). Über eine Wirkung des Vitamin A auf den Mineralaushalt. *Z. ges. exp. Med.* **89**, 771–779.

Clausen, S. W., McCoord, A. B., Baum, W. S., Steadman, C. G., Rydeen, J. O. & Breese, B. B. (1940). The determination of free and esterified Vitamin A in human blood and urine. *Amer. chem. Soc. Abstr.* 100th meeting.

Collazo, J. A. & Rodriguez, J. S. (1933). Hypervitaminose A. *Klin. Wschr.* **12**, 1768–1771.

Datta, S. P., Overell, B. G. & Stack-Dunne, M. (1949). Chromatography on alumina-impregnated filter paper. *Nature, Lond.*, **164**, 673.

Davies, A. W. & Moore, T. (1934). The distribution of vitamin A in the organs of the normal and vitaminotic rat. *Biochem. J.* **281**, 288–295.

Glover, J., Goodwin, T. W. & Morton, R. A. (1947). Studies in vitamin A. 2. The relationship between blood vitamin A levels and liver stores in rats. *Biochem. J.* **41**, 97–100.

Hoff, F. & Jeddeloh, B. (1934–5). Experimentelle Untersuchungen mit Epithelkörperhormon und mit Vitamin A. *Z. ges. exp. Med.* **95**, 67–76.

Irving, J. T. (1949). The effects of avitaminosis and hypervitaminosis A upon the incisor teeth and incisal alveolar bone of rats. *J. Physiol.* **108**, 92–101.

Mellanby, E. (1938). The experimental production of deafness in young animals by diet. *J. Physiol.* **94**, 380–398.

Mellanby, E. (1941). Skeletal changes affecting the nervous system produced in young dogs by diets deficient in vitamin A. *J. Physiol.* **99**, 467–486.

Mellanby, E. (1944). Croonian lecture: nutrition in relation to bone growth and the nervous system *Proc. roy. Soc.* B, **132**, 28–46.

Mellanby, E. (1946). Vitamin A and bone growth: the reversibility of vitamin A deficiency changes. *J. Physiol.* **105**, 382–399.

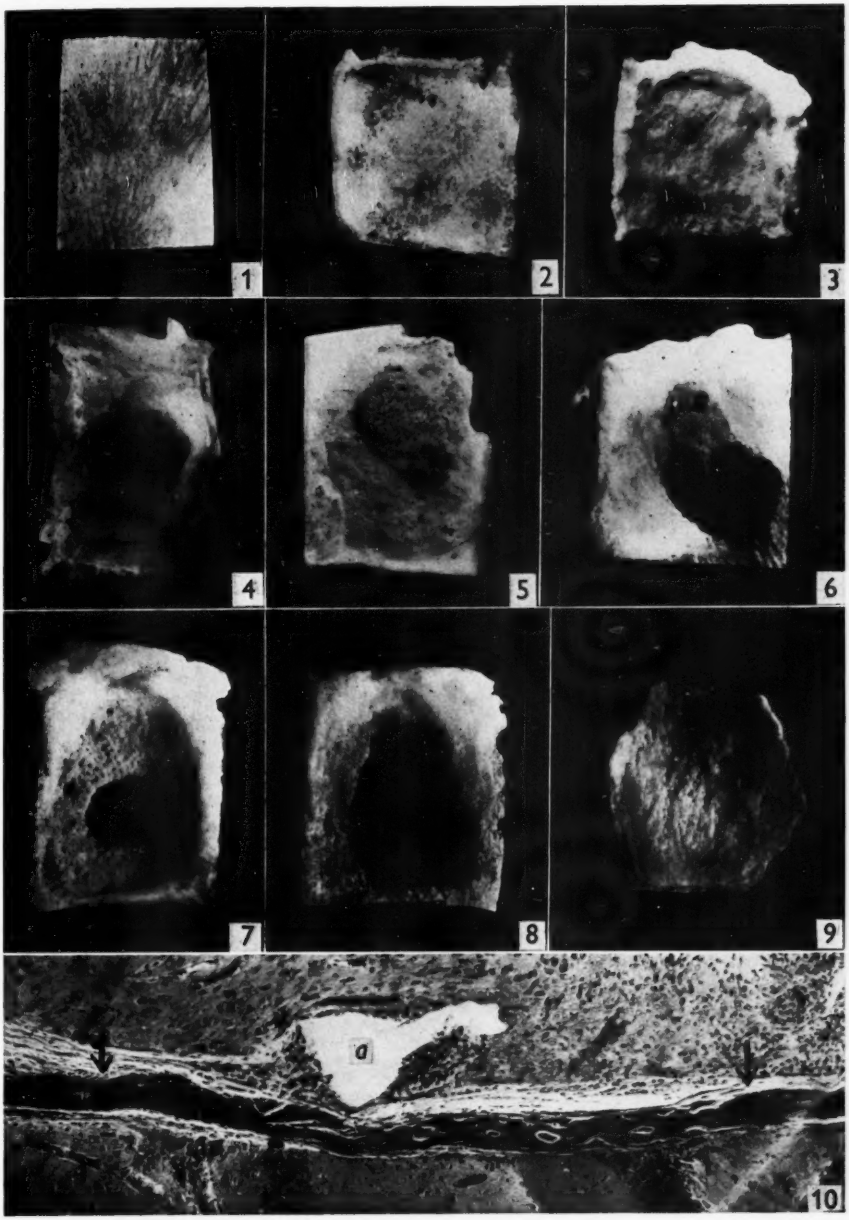
Moore, T. & Wang, Y. L. (1945). Hypervitaminosis A. *Biochem. J.* **39**, 222–228.

Rodahl, K. (1949). The toxic effect of polar-bear liver. *Norsk. Polarinst. Skr.* **92**, 1–90.

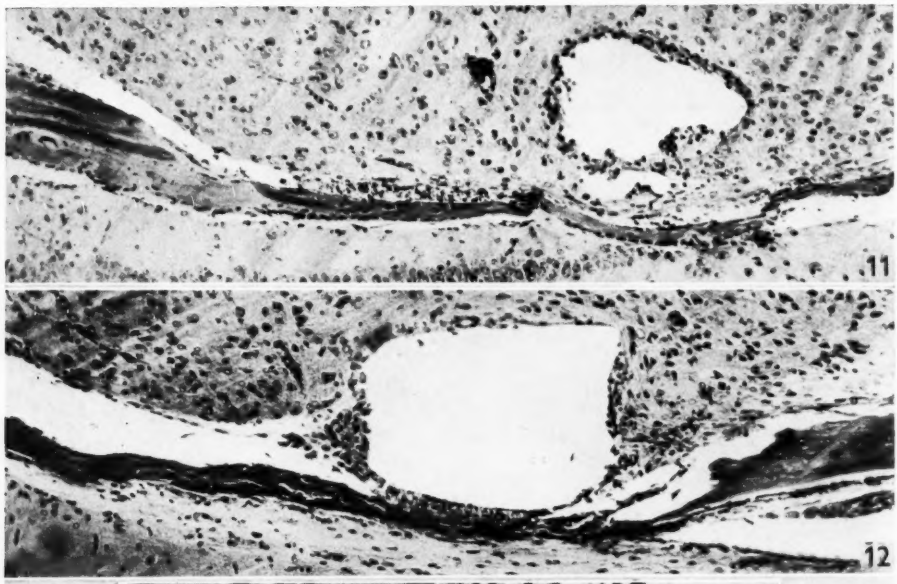
Strauss, K. (1934). Beobachtungen bei Hypervitaminose A. *Beitr. path. Anat.* **94**, 345–352.

Takahashi, K., Nakamiya, Z., Kawakami, K. & Kitasato, T. (1925). On the physical and chemical properties of biosterin (a name given to fat-soluble A) and on its physiological significance. *Sci. Pap. Inst. phys. chem. Res., Tokyo*, **3**, 81–145.

Vedder, E. B. & Rosenberg, C. (1938). Concerning the toxicity of vitamin A. *J. Nutrit.* **16**, 57–68.



BARNICOT—THE LOCAL ACTION OF VITAMIN A ON BONE


11
12

a
b
13

a
b
14

WALKER, S. E., EYLENBERG, E. & MOORE, T. (1947). The action of vitamin K in hypervitaminosis A. *Biochem. J.* **41**, 575–579.

WESLAW, W., WRONSKI, B., WROBLEWSKI, A. & WROBLEWSKI, B. (1938). Symptomologie und Verlauf der A-hypervitaminose bei Ratten infolger enteraler, subcutaner und percutaner Darreichung von Vitamin-A Konzentraten. *Klin. Wschr.* **17**, 777–781.

WOHLBACH, S. B. (1947). Vitamin A deficiency and excess in relation to skeletal growth. *J. Bone Jt Surg.* **28**, 171–192.

WOHLBACH, S. B. & BESSEY, O. A. (1941). Vitamin A deficiency and the nervous system. *Arch. Path. Lab. Med.* **32**, 689–722.

EXPLANATION OF PLATES

PLATE 1

Figs. 1–9. Macerated grafts showing, in the majority of cases, the structure of the endocranial surface on which the chemical implant lay. The edge nearest the skull-wound is uppermost. ×16.

Fig. 1. Ungrafted piece of parietal bone cut from the antero-lateral region of a 10-day-old animal.

Fig. 2. No chemical implant; graft removed after 14 days. Irregular thickening of the surface.

Fig. 3. β-Carotene pellet; graft removed after 14 days. Irregular thickening of the surface.

Fig. 4. Powdered vitamin A + cholesterol; removed after 14 days. Slightly sunken area of thin bone with a few perforations in its central region; thickening of the peripheral region of the graft with the original thickness of the marginal bone showing in some places. Compare plot of osteoclasts, Text-fig. 2.

Fig. 5. Powdered vitamin A + cholesterol; removed after 14 days. Slightly sunken, rounded area of thin, sieve-like bone showing fine canals leading from the foramina. Compare plot of osteoclasts on this bone, Text-fig. 3.

Fig. 6. Vitamin A, single fragment; removed after 14 days. The convex ectocranial surface is seen in this specimen. A large single perforation with a tongue of thin bone remaining at one side.

Fig. 7. Powdered vitamin A + oestradiol; removed after 14 days. There is a large area of sieve-like bone perforated in the centre; the upper edge is very much thickened. Compare plot of osteoclasts on this bone, Text-fig. 4.

Fig. 8. Vitamin A + cholesterol; removed after 14 days. There is a large perforation with a margin of thin bone. Compare plot of osteoclasts on this specimen, Text-fig. 5.

Fig. 9. Vitamin A, single fragment; removed after 14 days. The edges of the specimen have been broken after maceration and the bone is considerably distorted by curling. In the central region are several small areas of commencing resorption. Compare the plot of osteoclasts, Text-fig. 1.

Fig. 10. Section of a graft of a single fragment of vitamin A, removed after 7 days. The central region opposite the vitamin (*a*) is composed of spongy bone which is distinct from the bone at the two sides. Osteoclasts are numerous on the surface nearest the vitamin between the points marked by arrows. Compare the projection drawing of this specimen, Text-fig. 6. ×125.

PLATE 2

Fig. 11. Section of a graft of a single fragment of vitamin A, removed after 14 days. On the left side the bone is thickened by new deposit. The central region on either side of the vitamin is formed of thin bone. The vitamin residue consists of two pieces, both rounded in form. Compare the projection drawing of this specimen, Text-fig. 7. ×125.

Fig. 12. Section of a graft of a single fragment of vitamin A, removed after 14 days. Opposite the vitamin residue and for some distance on the left-hand side of it the bone is thin and is lined by numerous osteoclasts on the surface nearest the vitamin. ×125.

Fig. 13. High-power field of the bone adjacent to the vitamin implant (*a*) in Pl. 1, fig. 10. Near the vitamin are macrophages. The bone of the original graft is clearly distinguishable at (*b*) and is being attacked by an osteoclast. It is faintly demarcated by a cement line from a layer of new bone on its lower surface. ×210.

Fig. 14. High-power field of the bone adjacent to the vitamin implant (*a*) in the same graft as is shown in Pl. 2, fig. 12, but from an adjacent section. The space left by the vitamin fragment is surrounded by foamy macrophages and the nearby vessels are somewhat congested (*b*). The bone layer shows numerous osteoclasts on the surface nearest the vitamin; it contains living osteocytes and has deeply staining osteoblasts on its lower surface. ×210.

THE DEVELOPMENT *IN VITRO* OF THE MAMMARY GLANDS OF THE MOUSE

By MARGARET H. HARDY
Strangeways Research Laboratory, Cambridge, and Department of Zoology, University of Cambridge

INTRODUCTION

During a study of the development of mouse-hair follicles *in vitro* (Hardy, 1949) it was noted that the mammary gland rudiments also grew and differentiated when explants were made from the ventral body wall of young embryos. In this paper a description is given of the development of mammary glands which took place in these experiments. Maximow (1925) reported growth of epithelium and connective tissue cells in explanted mammary gland fragments from adult rabbits. Balinsky (1950*b*) obtained a little differentiation of the mammary rudiments of mouse embryos in tissue culture, but no previous reports of an advanced degree of mammary gland differentiation *in vitro* are known to the author.

MATERIAL AND METHODS

Three experiments* were performed with explants from the ventral body wall of 10- to 13-day embryos. The strains of mice used were (1) C57 Black, (2) albino (laboratory stock), and (3) grey-bellied agouti (mixed stock) respectively. The explants used in Exps. 1 and 3 were right or left halves of the ventral body wall, and each included the loci of two to five mammary glands; those used in Exp. 2 included both sides of the ventral body wall, and each contained the loci of three to five pairs of glands. No attempt was made to distinguish the sexes in these embryos. All the explants were cultivated by the watch-glass method described by Fell & Robison (1929), in a mixture of equal parts of adult cock plasma and chicken embryo extract; the procedure has already been described (Hardy, 1949). Pieces of skin from the mammary regions of litter-mates to the experimental embryos were fixed at the same time to serve as controls; material was obtained from other 12- to 19-day embryos and from young mice 4 days after birth for the study of normal development of mammary glands. All sections were cut at 8μ and stained with haematoxylin and chromotrope 2*R*.

As in the previous paper (Hardy, 1949) the pre-natal ages are defined in days from the appearance of the vaginal plug, and the post-natal ages in days from birth prefixed by a 'plus' sign.

THE EARLY STAGES OF NORMAL DEVELOPMENT OF MAMMARY GLANDS

The formation of mammary glands in embryos and young mice was described by Turner & Gomez (1933) and re-examined by Balinsky (1950*a*, *b*). The following brief account is based on that of Turner & Gomez. Five pairs of mammary buds

* The experiments described in this paper as 1, 2 and 3 are identical with Exps. 10, 11 and 12 respectively in the series for which hair-follicle development was described (Hardy, 1949).

appear as lentoid thickenings in the epidermis of embryos of about 12 days' gestation. The buds become rounded and remain connected with the rest of the epidermis by a short neck. They increase slowly in size until in the 17- or 18-day embryo a solid outgrowth, the primary sprout, appears at the distal extremity of the bud. A canal begins to form at the distal end of the sprout one day later and secondary sprouts develop from the primary one at about 20 days. At +1 day a streak canal develops from the original mammary bud by keratinization of cells in its centre.

Development of the duct system is most rapid during the first week after birth. Canals begin to appear in the secondary sprouts during the first day, and in the next 6 days tertiary and quaternary sprouts develop and the canal-formation in primary and secondary sprouts becomes more complete. From the second week until puberty, at about +6 weeks, development is again slow, and includes only an increase in the length and number of ducts. Alveoli are not formed before puberty.

Turner & Gomez found that in female embryos an epithelial ingrowth from the epidermis begins at 18 days at the circumference of a circle surrounding the mammary bud; this is the rudiment of the nipple. During the first week after birth the ingrowth extends deeper into the derma, while hair follicles develop outside but never inside the circle. Eversion of the nipple does not take place until 6 weeks after birth. In male embryos, according to Turner & Gomez, the primary sprout becomes detached from the epidermis at the nineteenth day, but otherwise the pre-natal development of the bud and sprouts is very similar to that of females. Secondary sprouts appear in males, but the duct system ceases to develop a few days after birth, and epithelial ingrowths and nipples are not formed. However, Richardson & Cloudman (1947) have pointed out that in many strains of mice, including C57 Black, there may be a considerable development of the duct system of males after birth.

Original observations were made on a few specimens to check some of the above findings. In a series of five embryos aged 10–13 days no indication was found of the mammary lines and folds which are characteristic of many other species, and the mammary buds seemed to arise from the unmodified epidermis and to be unconnected with each other (Pl. 1, fig. 1). Balinsky also failed to find mammary lines, although they were reported by Turner & Gomez. In the present study a bud and a solid primary sprout were found in a female 19-day embryo of the C57 Black strain. A +4-day female of the same strain showed canalized primary and secondary ducts (Pl. 1, figs. 2, 3) and a well-developed epithelial ingrowth (Pl. 1, fig. 3). A male litter-mate killed at the same time had many canalized ducts (Pl. 1, fig. 4), but no connexion between the ducts and the epidermis was found.

OBSERVATIONS ON THE DEVELOPMENT OF MAMMARY GLANDS *IN VITRO*

Some development of mammary tissue took place *in vitro* in most of the explants of the ventral body wall of mouse embryos. From one to four mammary glands differentiated in each explant, and the growth of the buds and sprouts was observed daily in the living cultures. The glands continued to grow until the cultures were fixed after 4–25 days *in vitro*. From Table 1 it may be seen that in thirteen out of seventeen explants some differentiation of mammary glands took place during cultivation, and its occurrence was confirmed by histological examination.

Table 1. *Summary of results*

Exp. no.	Strain of mice	Age of embryos (days)	No. of healthy explants of ventral body wall	No. of explants showing mammary differentiation *in vitro*	Age when primary sprout formed (days)
1	C 57 Black	13	10	8	17–20
2	Albino	10	3	1	18
3	Agouti	12	4	3	18

In Exp. 2 small mammary buds appeared in the explants from 10-day embryos during the first few days *in vitro*; in the other experiments buds were already present at the time of explantation. The buds enlarged slowly for several days (Pl. 1, fig. 5) and then solid primary sprouts developed, at about the same time as in normal development (Table 1). While the canals of the primary sprouts were being formed, secondary sprouts appeared and then they too began to show a lumen (Pl. 1, fig. 6). This stage was reached at about +1 day in the young mouse and at about 21 days in the explants. Although the growth of the original bud was slow for the first 6 days after its appearance, the glands as a whole grew relatively fast as soon as the first sprout was formed, i.e. a number of branches developed within a short time and they appeared to grow faster than the original bud. The new buds quickly began to differentiate.

The subsequent differentiation of mammary glands *in vitro* was similar to that in mice during the first week after birth. Tertiary and later sprouts were formed, and canalization of all the ducts continued so that eventually there was a complete system of canals through the branches of each gland. The primary sprout usually had a wide lumen and walls about three or four cells thick (Pl. 2, figs. 7, 8). The later sprouts had a smaller lumen and thinner walls (Pl. 2, fig. 7). In most glands the original mammary bud became traversed by a streak canal into which the primary duct opened (Pl. 2, figs. 8–10). Mammary glands continued to branch and grow until the explants were fixed. Even after 25 days *in vitro* mitoses were found in the sprouts.

The secondary and later ducts were lined by a single layer of cuboidal epithelium, and surrounded by a second epithelial layer (Pl. 2, figs. 9, 11). Outside this was connective tissue, arranged in the normal manner—first in concentric circles around the ducts as the mantle layer, and then in a more irregular fashion between the ducts as the gland stroma (Pl. 2, figs. 8, 11). Some solid material was observed in many lumina in sections of fixed tissue; this may be the 'duct secretion', which is said to be of widespread occurrence in mammary glands of various animals at all stages of development (Richardson, 1947). No true alveoli were formed in the explants, unless the two-layered terminal branches may be considered as the 'resting alveoli' described by Engel (1947).

One explant from a 10-day embryo showed the greatest number of stages of mammary gland differentiation which have been produced in a single tissue culture. From the ventral half of the trunk, which showed no trace of mammary rudiments, were developed a bud, primary, secondary and later sprouts and canals, a streak canal and a connective tissue sheath (Pl. 2, figs. 10, 11).

The orientation of the glands with respect to the skin surface *in vitro* was similar

to that *in vivo*; mammary buds grew at right angles to the skin surface, primary sprouts at an acute angle and later sprouts mainly in directions parallel to the skin surface (Pl. 2, fig. 9).

The epithelial ingrowth which is formed in female embryos did not usually develop *in vitro*. One explant showed a small ingrowth which half surrounded a mammary gland and which might be homologous with the normal structure. In most explants, however, the mammary buds were surrounded by a circular area of epidermis in which hair follicles did not develop and which may perhaps represent a 'nipple area' where follicle-formation is inhibited.

At the time of explantation no attempt was made to distinguish between the sexes in the donor embryos, and therefore a study of the expression of sex differences was not possible. Nearly all the glands showed some form of canal opening on to the skin surface, but there were differences of canal shape and structure, as may be seen by a comparison of Pl. 2, figs. 8–10. However, there was no nipple development. From the present limited number of observations it was impossible to divide the glands which developed *in vitro* into male and female types. Obviously this question requires further research.

DISCUSSION

The experiments have shown that mammary glands can undergo many stages of differentiation *in vitro*, and that they can be produced even from the histologically undifferentiated epidermis and derma of 10-day mouse embryos. The rate of differentiation in explants was comparable with that in the normal animal, and growth of the glands could be observed for as long as 25 days *in vitro*.

Balinsky (1950*a*) found that in mouse embryos during the first 5 days after the appearance of the mammary bud the growth rate of the bud was lower than that of the adjacent tissue or of the body as a whole. The formation of a primary sprout marked the beginning of a new phase of very active growth at a rate which surpassed that of the rest of the body. It is interesting to note that the period of slow growth which Balinsky described as the 'diapause' occurred also *in vitro*, and usually lasted for about 6 days; it was regularly followed by a period of more rapid growth and differentiation once sprouting had started. These facts suggest that the pre-natal and early post-natal development of mammary glands is not entirely dependent on hormonal or other stimuli from other parts of the body. It seems that even as early as the tenth day of gestation the ventral skin is determined for bud-formation, a 'diapause' and a sudden increase in growth and differentiation. Not even the timing of these events was seriously disturbed by the conditions of cultivation. It is worthy of note that mammary glands, like hair follicles (Hardy, 1949) can grow and differentiate in a medium that is entirely avian in origin.

It seems that this simple tissue culture method could be used to advantage to study such questions as the sensitivity of mammary glands to hormones at the time of birth or the relative importance of genetic and hormonal influences on early gland development.

SUMMARY

1. Mammary glands developed *in vitro* in explants of the ventral body wall of embryonic mice; the tissue was cultivated in watch-glasses in a mixture of adult cock plasma and chicken embryo extract.

2. Explants from 10-, 12- and 13-day embryos were cultivated for up to 25 days *in vitro*, and primary, secondary and later mammary ducts and a streak canal were formed. The gross structure, histological details, and rate of differentiation were similar to those in embryos and young mice, and the final stage reached was similar to that in a 7-day-old mouse.

3. The experiments *in vitro* suggest that the early stages of mammary development in the mouse are not primarily dependent on specific hormonal stimulation or other influences from different parts of the body.

These observations were made during the tenure of an Overseas Studentship of the Council for Scientific and Industrial Research (Australia) (now the Commonwealth Scientific and Industrial Research Organization). I am indebted to Dr H. B. Fell and Prof. J. Gray for providing facilities at the Strangeways Research Laboratory, Cambridge, and the Zoological Laboratory, University of Cambridge, respectively. I wish to acknowledge with gratitude the encouragement and advice of Dr H. B. Fell and Dr A. Glucksmann, of the Strangeways Research Laboratory and of Dr B. I. Balinsky of the Institute of Animal Genetics, Edinburgh. Mr V. C. Norfield and Mr G. Lenney kindly took the photographs.

REFERENCES

Balinsky, B. I. (1950*a*). On the prenatal growth of the mammary gland rudiment in the mouse. *J. Anat., Lond.*, **84**, 227–235.

Balinsky, B. I. (1950*b*). Histological studies on the development of the mammary gland. *Proc. roy. Soc. Edin.*, B. (in the press).

Engel, S. (1947). Discussion on some recent developments in knowledge of the physiology of the breast. *Proc. R. Soc. Med.* **40**, 899–900.

Fell, H. B. & Robison, R. (1929). The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated *in vitro*. *Biochem. J.* **23**, 767–784.

Hardy, M. H. (1949). The development of mouse hair *in vitro* with some observations on pigmentation. *J. Anat., Lond.*, **83**, 364–384.

Maximow, A. (1925). Über krebsähnliche Verwandung der Milchdrüse in Gewebskulturen. *Virchows Arch.* **256**, 813–845.

Richardson, K. C. (1947). Some structural features of the mammary tissues. *Brit. med. Bull.* **5**, 123–129.

Richardson, F. L. & Cloudman, A. M. (1947). The mammary gland development in male mice at nine weeks of age. *Anat. Rec.* **97**, 223–237.

Turner, C. W. & Gomez, E. T. (1933). The normal development of the mammary gland of the male and female albino mouse. *Bull. Mo. agric. Exp. Sta. Res.* **182**, 3–43.

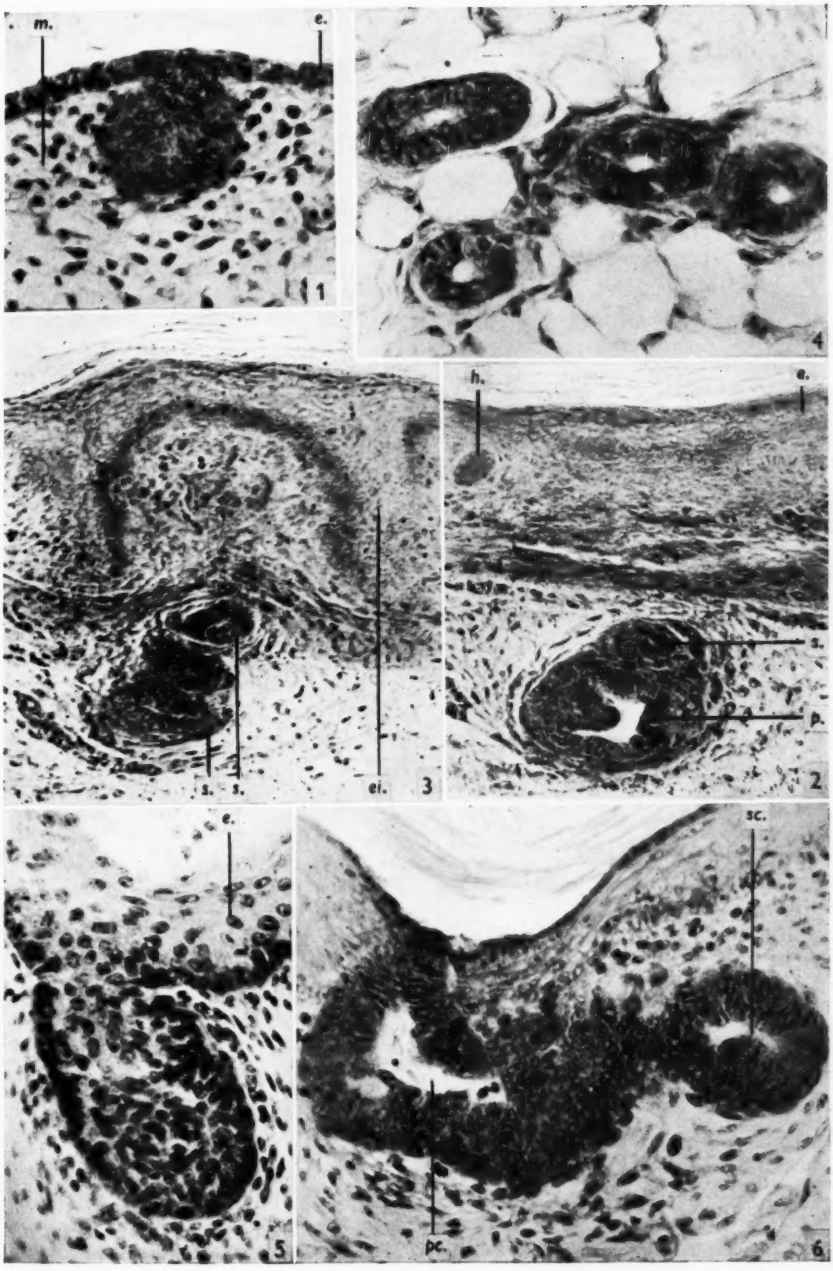
EXPLANATION OF PLATES

e., epidermis; *ei.*, epithelial ingrowth; *h.*, hair follicle; *m.*, mesoderm; *ma.*, mantle layer; *p.*, primary sprout; *pc.*, primary canal; *s.*, secondary sprout; *sc.*, secondary canal; *st.*, streak canal; *tc.*, tertiary canal.

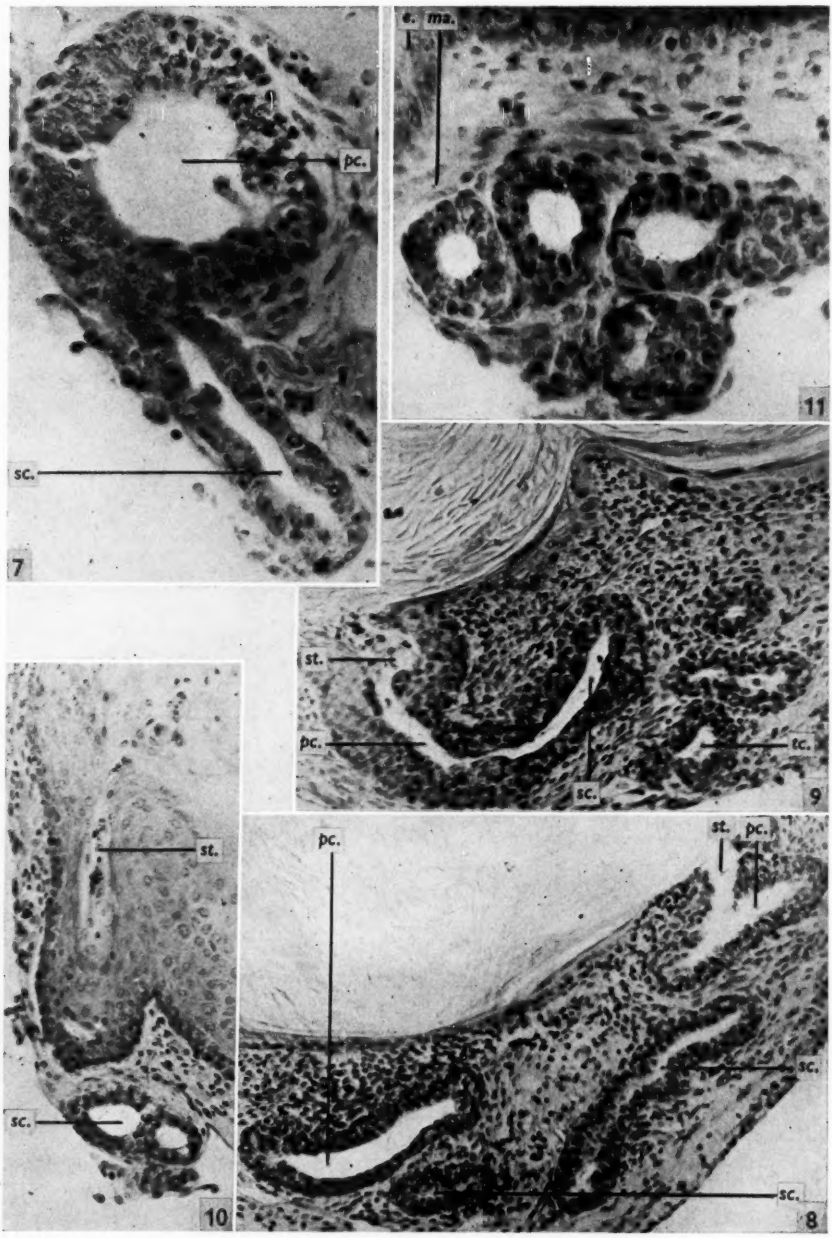
Plate 1

Fig. 1. Mammary bud in 12-day mouse embryo (control to Exp. 3). ×350.

Fig. 2. Section through part of mammary gland of +4-day female mouse, showing primary and secondary canals. ×200.



HARDY—DEVELOPMENT *IN VITRO* OF THE MAMMARY GLANDS OF THE MOUSE



HARDY—DEVELOPMENT *IN VITRO* OF THE MAMMARY GLANDS OF THE MOUSE

Fig. 3. Section through another part of the gland shown in fig. 2, showing secondary canals and epithelial ingrowth. ×200.

Fig. 4. Secondary canals, embedded in adipose tissue, of mammary gland of +4-day male mouse. ×400.

Fig. 5. Mammary bud in an explant from 13-day mouse embryo, enlarged after 4 days *in vitro* (Exp. 1; compare fig. 1). ×350.

Fig. 6. Primary and secondary canals of mammary gland in explant from 12-day embryo after 9 days *in vitro* (Exp. 3). ×350.

PLATE 2

Fig. 7. Primary and secondary canals of mammary gland in explant from 13-day mouse embryo after 22 days *in vitro* (Exp. 3). ×200.

Fig. 8. Two adjacent mammary glands in explant from 12-day mouse embryo after 18 days *in vitro* (Exp. 3). ×200.

Fig. 9. Differentiated mammary gland in explant from 12-day embryo after 18 days *in vitro* (Exp. 3). ×200.

Fig. 10. Secondary and streak canals of mammary gland in explant from 10-day embryo after 18 days *in vitro* (Exp. 2). ×200.

Fig. 11. Secondary canals of mammary gland from 10-day embryo after 18 days *in vitro* (Exp. 2; compare fig. 4). ×400.

ANATOMICAL NOTE

INVASION OF NERVE HOMOGRAFTS BY REGENERATING MUSCLE FIBRES

By F. K. Sanders, *Department of Zoology, University of Oxford*

In a study of the fate of nerve homografts in the rabbit (Sanders, 1949), several cases were noted in which regenerating muscle fibres were found invading the Schwann tubes of degenerated nerves. For example, in one rabbit in which a tibial nerve homograft 6·5 cm. long had been used to fill a gap in the peroneal nerve in the thigh, the graft being fixed in place with autologous plasma, it was found at biopsy 30 days later that the proximal junction of the graft was strongly adherent to the underlying muscle, and could only be separated from it with difficulty.

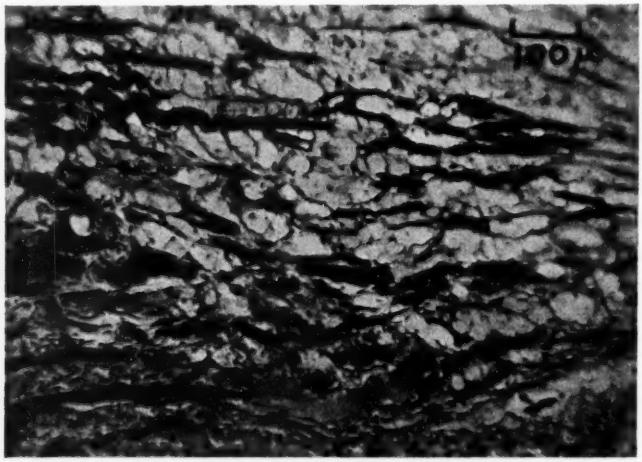


Fig. 1. Longitudinal section of the proximal end of a nerve homograft containing regenerating muscle fibres. The proximal junction of the graft lies to the left of the photograph. (1881 f. 6. 3, Masson stain.)

On histological examination it was seen that a group of muscle fibres was adherent to one side of the proximal junction, and that from it bundles of regenerating muscle fibres passed distally within the Schwann tubes of the nerve graft (Text-fig. 1). In the serial sections these muscle bundles were found to extend for about 5 mm. beyond the junction. The nerve graft itself appeared to be dead, there being very few Schwann cells or macrophages visible. However, the collagenous walls of the endoneurial tubes were preserved, and there was a considerable round-cell infiltration of the two junctions.

Such invasion of nerve by muscle fibres has only been found in nerve homografts, and only after intervals sufficient to ensure the destruction of the native cell population of the graft. Evidently, when depleted of their cell content, endoneurial tubes are able to act as a support for the extension of regenerating muscle fibres in a similar manner to the endomysial tubes of a muscle itself (Le Gros Clark, 1946). The phenomenon, however, does not seem to occur in nerve autografts, which indicates that muscle fibres are not able to enter the endoneurial tubes when these are packed with Schwann cells and macrophages.

REFERENCES

Le Gros Clark, W. E. (1946). An experimental study of the regeneration of mammalian striped muscle. *J. Anat., Lond.*, **80**, 24–36.

Sanders, F. K. (1949). The fate of nerve homografts in the rabbit. *J. Anat., Lond.*, **83**, 80.

Portrait by Messrs Blank and Stoller of Montreal

SAMUEL ERNEST WHITNALL
1876–1950

IN MEMORIAM

SAMUEL ERNEST WHITNALL, M.A., M.D., B.Ch. (Oxon.), M.R.C.S., L.R.C.P. (Lond.)

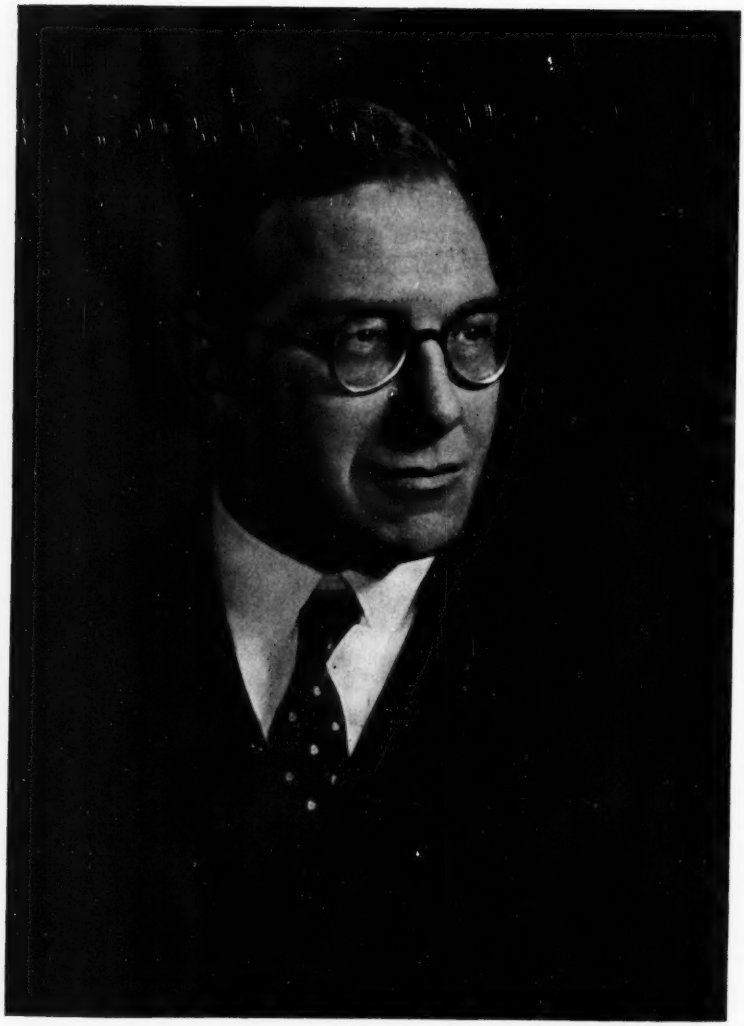
Samuel Ernest Whitnall was born on 30 March 1876, at Eccles in Lancashire, the son of Arthur W. Whitnall of Flixton. He received his early education at Radley College and afterwards became a student at Manchester University. From Manchester he went to Magdalen College, Oxford, where he was Welch Memorial Prizeman in 1904. His clinical years he spent at St Thomas's Hospital, and he qualified M.R.C.S., L.R.C.P. in 1908, taking the M.D., B.Ch. degrees of Oxford in the next year. From the time of his qualification until the outbreak of the 1914 war, he was Demonstrator of Human Anatomy under Prof. Arthur Thomson at Oxford. During his period of teaching at Oxford he became Radcliffe Prizeman in 1913 and, working with Thomson, laid the foundation of his future work on ophthalmological anatomy. He joined the Anatomical Society in 1910 and during the next two years made some of his most important contributions on the anatomy of the orbit to the *Journal*. Notable among these are the papers: 'On a ligament acting as a check to the action of the levator palpebrae superioris muscle' (Vol. 45, p. 131); 'On a tubercle on the malar bone, and on the lateral attachment of the tarsal plate' (Vol. 45, p. 426); and 'The retractor bulbi muscle in Man' (Vol. 46, p. 36).

On the outbreak of war, Whitnall took a commission in the R.A.M.C., and in 1915 was appointed junior surgeon at the 3rd Southern General Hospital and subsequently he spent nearly three years in France as Medical Officer, Oxford Hussars, and the 2nd Cavalry Field Ambulance. In 1918 he acted as Administrator to the Orthopaedic Hospital, Oxford. On demobilization, at the end of the war, he returned to his old love and became again a teacher of Anatomy. In 1919 he was appointed Robert Reford Professor of Anatomy at McGill University and spent the next 15 years in Canada. Here he continued his studies on ophthalmic anatomy, and in 1921 the *Anatomy of the Orbit* was published. This work will always rank as Whitnall's memorial as far as anatomy is concerned. Its merits were recognized in the award of the Nettleship Prize in 1923, and it still maintains its place as a book of reference for all details of the anatomy of the orbit and its contents. In 1922 there was published in Montreal a little brochure of only 46 pages and issued in wrappers. Its title was *The Study of Anatomy* and it was written as a student's guide—not to dissection or to details of human structure, but to the attitude to be adopted towards anatomy as a part of the medical curriculum and as a cultural exercise. It was a most attractive and extremely sane piece of writing and fortunately it has received more assurance of ultimate survival by the publication, in London, in 1933 of an enlarged edition of 93 pages bound in cloth. To this edition is appended a bibliography of general reading after the manner of Raymond Pearl's 'To begin with'.

To write of Whitnall the anatomist is to tell only half the story. Medical students know him as the describer of the tubercle on the malar bone to which the lateral attachment of the tarsal plate is made; for the name 'Whitnall's tubercle' became

rapidly incorporated into eponymous anatomical nomenclature. But the older generation of anatomists will remember him—and always be happy in their memories—as 'Tingle'. In the guise of Tingle he stands out as the man who had to perfection that gift of typically English humour perpetually enshrined in the pages of *Punch*, a journal to the columns of which he made many characteristic contributions. Members of the Anatomical Society who attended the Society's dinners in the early years of the twentieth century will never forget the anatomist Whitnall who, at the appropriate moment, became Tingle the humorist and incomparable after-dinner entertainer. Unfortunately, there can be no more than may be counted on the fingers of one hand who remember Tingle as an interpreter of typical English humour, who made memorable many occasions in the life of our Society. There was no real conflict between Tingle the humorist and Whitnall the anatomist, for they collaborated harmoniously in the production, in 1913, of *Astonishing Anatomy*. This was a brochure of 86 pages, illustrated from the price list of the Army and Navy Stores and from other highly irrelevant sources. To the relief of one who assisted in its compilation and is still engaged in teaching students, it is a literary curiosity that is now but rarely met with. Although *Astonishing Anatomy* was not a best seller, it was never in danger of being misunderstood, since it was a typically English piece of foolery. But when Whitnall went to Montreal his particular brand of humour was not so well appreciated, and his little pamphlet entitled *The Architectonics of the Monogamal Oestrous Cycle in the Greater Pifflecock* (Gallinopunk gigas occidentalis) was regarded in certain academic circles with some disfavour. This publication, which has no date nor printer's imprint, was issued in 1932. It consists of 15 pages with humorous illustrations and is written under the pseudonym of 'Stodge D. Loquax of the Wishful Destitute of Anatomy. Pah.' A brief quotation from the Introduction will make clear the real purpose of the author in ridiculing the dodder growth of verbalism that entangles modern scientific writing: 'The present prospective preliminary study was made with a view to correlating the standardization of the effects of vestigial dissemination in co-parental dissimilarities, in so far as preconstructive concepts of our primordial principles permit and then some.' The skit was a salutary one, but it was not understood nor appreciated in its time and place and, judging by some more recent contributions to biological scientific literature, it has failed in its object of killing by ridicule. Samuel Butler recognized two forms of mystification; 'the mystification of absolute ignorance and the mystification of fogged science'. Unfortunately, the fogging of science by the development of verbal smoke-screens was not arrested by the sarcasm of Butler nor by the humour of Whitnall.

It was in 1935 that Whitnall returned to England to occupy the Chair of Anatomy at Bristol University, a post that he held until his retirement in 1941. Unfortunately his health was somewhat impaired and on 19 February 1950, within six weeks of his seventy-fourth birthday, there passed away the man who has the highest claim to be remembered as a 'human' anatomist, for above all Whitnall was that which in reality is a rare thing—a truly lovable human being. He is survived by his wife and two sons and a daughter and to them we would convey the sympathies of his fellow-members of the Society. F.W.J.



Elliott and Fry

ARTHUR BEENY APPLETON

IN MEMORIAM

ARTHUR BEENY APPLETON, M.A., M.D., M.R.C.S., L.R.C.P., F.Z.S.

Emeritus Professor of Anatomy, University of London

Arthur Beeny Appleton died in St Thomas's Hospital on 22 April 1950, at the age of 61 years. He had been a member of the Anatomical Society since 1919, and President of the Society from 1947 to 1949.

Educated at the City of London School, at Downing College, Cambridge, and at St Bartholomew's Hospital where he was a scholar, he graduated B.A. in 1910 and M.A. in 1913, and in 1914 became medically qualified with the conjoint diploma. During the 1914–18 war he served in the R.A.M.C., first in France and later in Macedonia. At this time his chief clinical interest was in orthopaedic surgery, but on his return in 1919 Appleton decided to specialize in anatomy and joined the staff of the Cambridge Department. He was subsequently appointed Lecturer (1926), a position which he held until he became Professor of Anatomy in the University of London at St Thomas's Hospital Medical School in 1934. In 1922 he was awarded the Horton-Smith Prize for his M.D. thesis, and in 1923 the Symington Memorial Prize in Anatomy. In 1930 he was elected to a Fellowship at Downing College.

The students who worked in the Cambridge Anatomy Department during the time that Appleton was there will have a vivid memory of the enthusiasm and freshness of outlook which he brought to the teaching of anatomy. His influence was felt especially by those who became interested in research, for Appleton was among the first to use experimental methods for the solution of anatomical problems in a British department. Perhaps owing to his earlier interest in orthopaedic surgery these methods were applied in investigations of the effects of posture and of mechanical factors in general on the growth of bone, and the results were published in the Proceedings of the Anatomical Society between 1922 and 1925.

When Prof. J. T. Wilson came to Cambridge in 1920, he gave a new impetus to the teaching of anatomy for the Natural Science Tripos, and this appealed very much to Appleton. With characteristic enthusiasm Appleton undertook responsibility in those parts of the course which dealt with adult vertebrate morphology, and soon became an authority on comparative myology and on problems associated with the evolution of the vertebrate muscular system. The practical class, in which vertebrate types ranging from cyclostomes to mammals were dissected, became a unique feature of the course. The experience gained led in 1929 to the publication of *A Guide to Vertebrate Dissection for Students*, based essentially on the work done in the Tripos course at Cambridge. It was also during this period that Appleton became interested in 'living anatomy', an interest partly inspired by Dr A. C. Barclay who was then in charge of the radiological department in the Medical School at Cambridge. Later, work in this field bore fruit in the publication, in collaboration with W. J. Hamilton and I. C. C. Tchaperoff, of *Surface and Radiological Anatomy*, first in 1938, with new editions in 1946 and 1949.

In 1934 Appleton took up his appointment at St Thomas's Hospital Medical School; in the reorganization of the Department an X-ray plant was installed so

that the student could be introduced at an early stage to the normal radiological appearance of anatomical structures. As a result Appleton became particularly interested in movements of the diaphragm and expansion of the lungs. This led next to an examination of the distribution of the bronchial tree and its variations and of the interrelationship of the different broncho-pulmonary segments, particular attention being paid to the distribution of the blood vessels in the segments. The results of these investigations were published in the *Journal of Anatomy*, *St Thomas's Hospital Gazette* and other journals. Although much valuable information had been gained concerning the adult lungs, Appleton felt that variations might be explained if the development of the broncho-pulmonary system were investigated. Accordingly, he made a large number of reconstructions of the lungs at different stages of development and was actively engaged in this work at the time of his illness.

Another result of Appleton's interest in 'living anatomy' and also perhaps of his earlier work on bone growth and muscle action, was the active part he took in the development of physical education. He was for a time a member of the advisory committee to the University of London on questions connected with the Diploma of Physical Education, and a University representative on the governing body of Chelsea College.

In addition to his professorship at St Thomas's, Appleton became Professor of Anatomy at the Royal College of Art, and in 1944 Hunterian Professor at the Royal College of Surgeons. Shortly after his retirement from the Chair at St Thomas's in 1948, he joined the staff of the new Institute of Animal Pathology which is being developed by the Agricultural Research Council at Babraham near Cambridge. After visiting veterinary schools in the United States, where he obtained much useful information, he began work in temporary quarters at the Prosectorium of the Zoological Society of London on the surgical anatomy of farm animals. It was the last project he was to take up, and it is a matter for deep regret that its completion was prevented by his untimely illness, although results of value had already been obtained. It is a tribute to his courage and enthusiasm that during this illness, the nature and outcome of which were obvious to him, he continued this work almost till the end.

Appleton's work for the Anatomical Society is well known to his colleagues in this country. He was a member of the Editorial Committee from 1942 to 1945, he served on the Council, and as President he took a very active part in the preliminary work necessary for the organization of the International Congress to be held in July this year. Many of us will miss his presence there.

Beyond his professional interests Appleton was keenly interested in music and art; as a young man he was a pianist of exceptional ability, and later, when President of the Arts Society at St Thomas's, his water colours were exhibited on several occasions. By nature he was essentially reserved and modest, and closer acquaintance revealed a genial and friendly personality which endeared him to many students and colleagues. He was married in 1918 and is survived by his widow and a son and daughter, to whom we extend our deepest sympathy.

F.G.
W.J.H.

REVIEWS

The Vertebrate Body. By Alfred Sherwood Romer. (Pp. viii + 643; 363 figures; 8vo; 27*s.* 6*d.* net.) Philadelphia & London: W. B. Saunders Company.

Zoologists and anatomists are already in Prof. A. S. Romer's debt for his *Vertebrate Paleontology*, and he has now increased this debt by the publication of the present volume. It should be said at the outset that the book is planned and composed in a manner different from that with which teachers and students on this side of the Atlantic are familiar for elementary and intermediate works. Instead of going systematically through a selection of types representative of the different grades of vertebrate structure, the book is arranged from beginning to end on a comparative plan, organ by organ. Perhaps the best way of introducing the book to British readers is to give the directives which the author says that he has had constantly in mind.

In addition to the comparative method of treatment and the insistence on adequate illustration, the guiding principles were: the presentation of the facts against a proper palaeontological background and on a sound embryological foundation; the inclusion of histological data; and the consideration of function. It may be said at once that the author has reached the objectives which he set for himself, and the result is a most informative book.

There are, of course, some matters which might be said to be controversial, but that is not to say that the author is not right. G. R. DE BEER

Angewandte und topographische Anatomie. Ein Lehrbuch für Studierende und Ärzte. Von Gian Töndury. Zürich: Fretz und Wasmuth Verlag AG., 1949. S.Fr. 70 (geb.), 65 (brosch).

Prof. Töndury's new text-book of topographical anatomy is characterized by an admirable sense of proportion. In 395 well-printed pages of text, most of which have line diagrams, he has given a most succinct description of the anatomy of the human body and has included in it a reasonable background of its development and morphology. The plates, of which there are 134, are quite excellent. They are for the most part drawn from actual specimens by Paul Winkler, and those who have had the privilege of visiting the Anatomical Institute in Zürich will know how beautiful the original dissections are and how faithfully they have been drawn and reproduced. Included with the plates are a number of carefully selected and also well-produced skiagrams.

The arrangement of the text is rather unusual. The first section deals with the anatomy of the trunk. This is followed successively by sections on the back, the head and neck, and the extremities. Interspersed in the text are 235 relatively simple but most attractive line figures and diagrams. The presentation is such that, in the reviewer's opinion, a student would require some elementary introduction to anatomy before reading this text. On the other hand, however, it is an admirable book for revising topographical anatomy and is one that certainly will be used by a student in his clinical as well as in his preclinical period. The whole approach is one of the practical clinical importance and significance of anatomy. This pragmatic approach, is not at the expense of general principles which are often adumbrated and not infrequently explicitly stated.

There are certain gaps in the presentation which are, doubtless, a reflexion of the difference in approach of the Continental as opposed to the Anglo-Saxon anatomist. Thus there is practically no reference to histology. The detailed anatomy of the central nervous system is also avoided. On the other hand, many of the sub-sections are much fuller than is usual in English texts. This applies more particularly to the topography of the gastro-intestinal tract and to the relations of the spinal cord and the spinal nerves to the vertebrae and intervertebral foramina. Indeed the account of the relations of the spinal nerves to these foramina is the best the reviewer has come across.

The language problem, of course, makes Prof. Töndury's new book, apart from its figures, of little direct use to a British medical student. But a senior student, or a junior demonstrator, wishing to revise anatomy and, at the same time, to learn anatomical German will not be able to find a more useful, or a more attractive, volume. J. D. BOYD

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1949

The Annual General Meeting of the Society, for the Session 1949–50, was held on Friday 25 November 1949, in the Department of Anatomy, Charing Cross Hospital Medical School, London. Prof. J. Kirk (Vice-President) was in the Chair.

The following are the authors' abstracts of the papers read:

Observations on the seal ovary.

By R. J. Harrison. *Department of Anatomy, Charing Cross Hospital Medical School*

The histological changes occurring in the reproductive tract are being investigated in four species of seals from the Falkland Islands Dependencies, the Faroe Islands and from British coastal waters (Matthews & Harrison, *Nature, Lond.*, 1949). Examination of the adult ovaries has revealed the presence of numerous sub-surface crypts invaginated into the cortex of the ovary and lined by germinal epithelium. The epithelial cells surrounding the primary oocytes are directly continuous with those of the crypts. The crypts alter in their degree of development and in the activity of the epithelium during the reproductive cycle, being most developed in November in antarctic seals. The first evidence of crypt formation is seen in the gonad of a 16 cm. foetus of a Weddell seal. The ovaries of near-term foetuses also contain active crypts, and these ovaries are remarkable for their large size and for the presence of considerable interstitial tissue similar to that found in the ovary of the foetal mare (Cole *et al.*, 1933). Some weeks after birth the ovaries are smaller and the interstitial tissue has shrunk and become condensed. An extensive vacuolation of the luteal cells is noted during the period of probable delay in implantation, and which disappears some time during the development of the early somite stages.

The inferior vena cava in the seal.

By R. J. Harrison and H. H. Bentall. *Department of Anatomy, Charing Cross Hospital Medical School*

Burne (1910) described a pair of venous plexuses arising from the inferior vena cava of the seal just above the diaphragm and surrounded by folds of pleura. He also noted the presence of a sphincter about the inferior vena cava just below the openings of the plexuses, a structure which he first noted in the walrus in the preceding year. Further description of the anatomy of the venous plexuses and the sphincter in a number of seals will be given, both in the adult and in the embryo. The venous plexuses are shown to consist of three main portions intimately related to the pericardium and their presence is first noted in a 20 mm. embryo of a crab-eater seal. The caval sphincter appears about the same time and is separated from the diaphragm by a fibrous band except anteriorly where some fibres are continuous with those of the diaphragm. There is an extensive abdominal and peri-renal network of veins whose function is probably not associated with that of the kidney, but with the caval sphincter. These veins are well developed in the embryo at the 20 mm. stage. The possible functions previously assigned to the venous plexuses and the caval sphincter are discussed.

Congenital cystic eye in a 5 mm. human embryo.

By W. R. M. Morton *Department of Anatomy, Queen's University, Belfast*

A 5 mm. human embryo showing disorganization of the developing eye and associated anomalies of the brain is described. There is evidence of slight lens formation, and the invaginating optic cup, which is crenated, appears to be separated from the surface ectoderm by mesoderm. The optic stalk is highly crenated and quite unlike the normal for this stage of general development. The forebrain is small, very highly convoluted, and does not show the normal divisions of cerebral vesicles and diencephalon. It is surrounded by a considerable thickness of mesoderm. The neural ectoderm is separated from Rathke's pouch ectoderm by a thin but definite layer of mesoderm. The hindbrain, otocyst, spinal cord and

the remainder of the organs appear to be normal. The significance of the appearance described is discussed.

Regional differences in the oxygen consumption of the chicken embryo. By R. J. O'Connor. *The John Burford Carlill Pathological Laboratories, Westminster School of Medicine, Horseferry Road, London, S.W.* 1

By a modification of the Cartesian diver micromanometer the oxygen consumption of 0·1–0·3 cu.mm. of chicken embryonic tissue can be measured. The volume of the tissue is calculated by compressing it beneath the cover-glass in a cell counting chamber of known depth, and measuring the area covered. From the chicken embryo on the third day of incubation, blocks of tissue have been dissected containing somites, spinal cord, and notochord. The rate of oxygen consumption of such blocks of tissue has been measured at various segmental levels and expressed as cu.mm. oxygen consumed per cu.mm. tissue per hour. At the stages of 25–28 somites there is no significant difference between the various segments. At 30–35 somites differences in the rate of oxygen consumption develop at different levels. The significance of this change is discussed.

Sudomotor escape areas after thoraco-lumbar sympathectomies. By P. A. G. Monro. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

The electrical skin resistance method has been used to map the areas showing loss of sweating after paravertebral thoraco-lumbar sympathectomy (T. 4–L. 3). All patients examined have shown an escape belt around the lower abdomen extending down on to the front and inner sides of the thighs corresponding to dermatomes T. 12–L. 3. Intermediate sympathetic ganglia occur particularly on and around these nerve roots, and are believed to be the explanation of the retention of autonomic function in these areas. Examination of post-mortem material from one of these patients has shown numbers of these ganglia which had escaped sympathectomy. Some ganglia contain cells which show degenerative changes but many are apparently normal.

'Intermediate' lumbar sympathetic ganglia in the human foetus. By J. D. Boyd. *Department of Anatomy, London Hospital Medical College, Turner Street, London, E.* 1

The distribution and possible connexions of sympathetic ganglia in the course of the lumbar rami communicantes and in relation to the lumbar spinal nerves are described. Attention is drawn to Wrete's work on these ganglia and their terminology is discussed.

Methods employed in demonstrating the anatomy of the ear. By Oliver Gray. *Humewood, Gragswood Road, Haslemere, Surrey*

Casts of the labyrinth have been obtained by various methods since the middle of the last century. By employing one of the new acrylate substances a measure of success has been obtained whereby the otic labyrinth can be shown within the periotic (perilymph) space. More valuable, however, is the fact that the 'Perspex method' can be utilized to display in detail most of the gross anatomy of the ear. The structures within the temporal bone are fixed by the 'polymer' (perspex) and the surrounding bone is dissolved away by means of strong acid, thus revealing the anatomical relationships with fidelity.

A brief description of the technique was given and preparations of the human labyrinth together with its related structures were demonstrated.

The influence of geographical isolation on the teeth of the green monkey. By S. Zuckerman and E. H. Ashton. *Department of Anatomy, Medical School, Hospital Centre, Birmingham* 15

A detailed quantitative analysis has been made of the dental dimensions and indices and of the cranial characters of (*a*) a collection of skulls of green monkeys (*Cercopithecus aethiops*

sabaeus), brought together by Sir Frank Colyer from the island of St Kitts in the West Indies, and (*b*) a corresponding group of green monkey skulls collected in West Africa. The green monkey was introduced into St Kitts some 300 years ago; the African green monkey is the modern representative of its parent stock.

Comparison shows that: (*a*) the teeth of the green monkey in the West Indies are bigger than those of its present-day African cousin; (*b*) there has been a decrease in the variance of the linear dimensions of the teeth; and (*c*) there has been an increase in the occurrence of such dental abnormalities as malposition, numerical variations, and variations in the number of roots of the third molar. These changes can be explained as an effect of selection acting on the original genetic constitution of the stock of green monkeys which became established in the Caribbean.

The composition of muscle nerves. By V. S. V. FERNAND and J. Z. YOUNG. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

The nerves of the limb muscles of rabbits all contain large as well as small nerve fibres, the distribution of fibre size being bimodal. On the other hand, the infra-hyoid muscles, diaphragm and muscles of the face, larynx and tongue are innervated by nerves containing only medium-sized fibres with a unimodal distribution. These muscles with unimodal nerves all exert little tension but shorten considerably during contraction; they probably have fewer proprioceptors than the limb muscles. The nerves to the extrinsic eye muscles have large fibres and a bidomal distribution. There is no close correlation between nerve fibre size and colour or speed of contraction of muscle fibres, or motor unit size. The proximal limb muscles receive larger fibres than the distal.

The effect of activity on the blood flow in the spinal cord. By E. J. FIELD, J. GRAYSON and A. F. ROGERS. *Departments of Anatomy and Physiology, The University, Bristol* 8

Experiments were carried out on the rabbit under nembutal anaesthesia. The thermo-electric method of measuring blood flow was used. Fine copper-constantan couples were introduced into the lumbar cord and in many cases also into the thoracic cord. Abdominal temperature and carotid blood pressure were also recorded. Ordinarily under the conditions of the experiment, the temperature of the cord was some $\frac{1}{2}$–1° C. lower than that of the abdominal cavity.

On pinching the animal's heel with sufficient force to elicit a moderate withdrawal response a rise in temperature was recorded in the lumbar but not as a rule in the thoracic cord. To determine whether this rise in temperature was really due to an increased blood flow through the part, heated thermocouples of the Gibb (1933) type were employed in further experiments. The principle here is to keep the recording point (by electrical heating) at a temperature *above* that of the blood. Under these conditions an increased blood flow past the point should produce a fall in recorded temperature because of the greater cooling effect. This was found to be the case.

Further experiments showed that a number of factors influence the actual response obtained in any particular instance, and that the response itself is more or less localized to the activated neural segments. The mechanism whereby the change in blood flow is brought about may be humoral (e.g. increase in CO_2 tension in the blood), or nervous. Whilst it is certainly true that an increased CO_2 tension in the blood does produce an increased flow in the cord, there is nevertheless a nervous supply of at least the larger vessels within the central nervous system, and a morphological basis for vasomotor reflexes within the nervous parenchyma does exist.

A study of retrograde degeneration in the oculomotor nucleus of the rhesus monkey, with a note on a method of recording it. By R. WARWICK. *Department of Anatomy, The University, Manchester* 13

Doubt has sometimes been expressed as to the regularity and reliability of retrograde degenerative changes in the oculomotor nucleus of the rhesus monkey subsequent to

division of the nerve. Six monkeys were therefore submitted to unilateral intracranial division of the oculomotor trunk, the operation being carried out by Mr R. A. Bailey, neurosurgeon. After an interval of 10–11 days serial transverse sections of the midbrain were studied, after staining by toluidine blue, carbol thionin blue, Einarson's gallocyanin, and Borrel's methylene blue, the latter two methods being found most satisfactory. Clear and extensive chromatolytic and other changes were noted in all cases.

In order to compare the localization of the changes in the different midbrain series, low-power photographs of every tenth section were taken, including the entire oculomotor nucleus. Full-plate enlargements of these were used as charts during microscopic examination of the corresponding sections, normal and degenerating cells being marked on the photographs in contrasting colours. The photographs were then bleached, leaving a clear pattern of the nucleus and the areas of degeneration at successive levels. The results in the midbrains studied were in very close agreement. The method is now being used to study the effects of severing individual branches of the oculomotor nerve in the orbit.

The myelinization of the central nervous system of *Natrix sipedon*. By FRANCIS JAMES WARNER. *National Hospital for Diseases of the Nervous System, Queen's Square, London, W.C.* 1

Myelinization occurs first in the cervical region of the spinal cord of *Natrix sipedon*. The medical longitudinal bundle and the lateral vestibulospinal tract are the first tracts to become medullated in the cervical level of the spinal cord. The fasciculus cuneatus is medullated earlier than the fasciculus gracilis. The spino-cerebellar and spino-mesencephalic tracts are medullated in the spinal cord later than the descending tracts.

In the brain stem the medial longitudinal bundle, the vestibular nerve and the lateral vestibulo-spinal tract become medullated early. The dorsal tecto-bulbar tracts medullate sooner than the ventral tecto-bulbar tracts. The vestibulo-cerebellar and spino-cerebellar tracts do not reach the cerebellum until a fairly late stage (213 mm.). The lateral lemniscus is medullated and can be traced to the inferior corpora quadrigemina at the 188 mm. stage. The cochlear root of the eighth cranial nerve medullates earlier than in the opossum or cat (at the 163 mm. stage of *N. sipedon*). The optic tracts and the optic layer of the optic tectum do not become medullated until a quite late stage (238 mm.).

The fibre tracts of the spinal cord, medulla and midbrain in *N. sipedon* medullate in a similar order as in the cat, as noted by Tilney & Casamajor (1924), Langworthy (1929), and the sequence is also similar to that of the opossum as described by Langworthy (1928). Thus in *N. sipedon* as in marsupials and placental mammals the most ancient fibre tracts phylogenetically medullate earlier than the fibre tracts of recent development.

The intramural blood supply of the upper jejunum in man. A radiological study. By F. S. A. DORAN. *Department of Anatomy, The University, Manchester* 13

Having noted that there is an absence of detailed information about the intramural blood supply of the jejunum it was decided to re-investigate this subject using the technique of vascular radiography that has been developed in recent years by A. E. Barclay, at Oxford. It appeared likely that this method, particularly the micro-radiography, would enable much finer details to be demonstrated. Twelve specimens of the first loop of the jejunum in human adults were studied, and were injected with 20 % bismuth oxychloride to demonstrate the arteries down to the size of the arterioles, and with 10 % silver iodide to fill the finer vessels. These media were injected through a hypodermic needle the small bore of which is a convenient safeguard against the use of too much pressure. In general, the current descriptions regarding the larger vessels were confirmed, but microradiographs revealed additional details about the distribution and termination of the smaller vessels. It was found that the arterioles tend to be arranged in parallel rows, each row being contained within the fold of a valvula connivens. The relatively avascular nature of the antimesenteric border was confirmed, but it was shown to occur only in some bodies, not in all. The irregular nature of the circumferential anastomoses of the vasa recta was demonstrated and the poor nature of the longitudinal anastomosis shown. Ligation experiments were performed to test the efficiency of

these longitudinal anastomoses, and at the best they were capable of bridging a gap of only 2½–3 in., and in some specimens it was considerably less.

Alkaline phosphatase in the large intestine. By B. F. MARTIN. *Department of Anatomy, University College of South Wales and Monmouthshire, Newport Road, Cardiff*

In contrast to the quite detailed observations that have been made on the histochemical distribution of alkaline phosphatase in the small intestine, comments on the distribution of the enzyme in the large intestine have been brief and lacking in details; in some cases not even the species from which the specimens were taken is made clear.

In the present investigation, sections from the large intestine of seven species (rabbit, guinea-pig, rat, mouse, dog, cat, and man) were examined; tissues from the caecum, proximal colon, distal colon, and rectum being included in all cases except in the case of man, in which only the distal colon and rectum were examined.

Marked species variations were discovered, but the pattern of enzyme distribution was constant for any one species.

From every tissue studied, a section stained with haematoxylin and chromotrop was examined for the presence of a striated border. It was found that there is a close correlation between the appearance of the intestinal border and the phosphatase picture. When the striated border is well developed, there is a strong reaction for alkaline phosphatase at the border, and vice versa.

The results of the investigation should be useful in forming a link with physiological studies on absorption from the large intestine.

FEBRUARY 1950

An ordinary meeting of the Society, for the Session 1949–50, was held on Friday, 17 February 1950, in the Department of Anatomy, St Mary's Hospital Medical School, Paddington, London, W. 2. The President (Prof. M. F. LUCAS KEENE) was in the Chair.

The following are the authors' abstracts of the papers read:

Maturation phenomena in the osteoblast. By J. J. PRITCHARD. *Department of Anatomy, St Mary's Hospital Medical School, London, W. 2*

Early ossification centres in foetal rats have been studied with a view to determining the sequence of the cytological and histochemical changes which accompany the differentiation of active osteoblasts from their mesenchymal precursors. The distribution of glycogen, alkaline phosphatase, cytoplasmic basophilia, mitochondria and Golgi bodies has been studied. It has been found that the precursor cells first become filled with glycogen, and later show intense alkaline phosphatase activity. Glycogen storage diminishes as the cells enlarge and show increasing cytoplasmic basophilia, increasing numbers of mitochondria and hypertrophy of the Golgi apparatus. As bone matrix is laid down the cytoplasmic basophilia decreases markedly, but alkaline phosphatase activity remains intense.

Squamous cells of the upper alimentary tract and the menstrual cycle. By I. D. HENDERSON. *Department of Anatomy, Guy's Hospital Medical School, London, S.E. 1*

During the course of an investigation into the cytology of the gastric resting juice using a modified Papanicolaou's E.A. 36 stain, a chance observation led to a more detailed investigation in the female. Four normal females were examined throughout a menstrual cycle with regard to their basal temperatures, vaginal smears and smears made from the centrifuged deposits of the gastric resting juice obtained by Ryle's tube. The smears were obtained about the same hour approximately every 5 days throughout the cycle. The main cellular content of the resting gastric juice was found to be squames from the mouth, pharynx or oesophagus.

The criteria, advocated by Papanicolaou, in the various stages of the cycle were followed, but particular attention was paid to those of clumping, folding and curling of cells, and the

relative proportions of basophil and acidophil cells. No special stress was laid on leucocyte counts in view of the possible trauma in passing the Ryle's tube. Similarly, mucus, as a factor in determining the stage of the cycle, was not considered directly.

On the basis of the above criteria it would appear that the squamous cells of the upper alimentary tract in the human female show evidence of an endocrine rhythm comparable with that of the vaginal epithelium.

Arterio-venous anastomoses in the human stomach. By T. E. Barlow. *The Medical School, King's College, Newcastle upon Tyne* 1

The presence of arterio-venous shunts in the gastric mucosa was deduced by Barclay and Bentley in 1949.

Using double injection techniques anastomoses of the direct type, as described by Schumacher, have been demonstrated in the submucous connective tissue of stomachs removed from the cadaver and at operation. The connecting channel is never less than 30 μ in diameter in the fixed specimen and is usually 40–60 μ.

Microradiographic demonstration of tissues treated by metallic impregnation. By G. A. G. Mitchell. *Department of Anatomy, The University, Manchester* 13

Various attempts to obtain microradiographic detail culminated in the remarkable results obtained by Lamarque (1938) using highly complicated apparatus. Barclay (1947) described a simplified technique which, although it does not give detail comparable to the best obtained by Lamarque, yet provides good results. Apart from demonstrating metallic deposits in a lung and leaves, Barclay used his method almost entirely for the elucidation in of fine vascular details following injection of radio-opaque solutions into vessels.

One had the idea that his method might be employed to investigate the arrangement of fibres having a selective affinity for metallic salts. In particular one hoped to obtain a more accurate idea of the pattern of intrinsic innervation in various glands, a subject still shrouded in considerable obscurity. Unfortunately, all the metallic techniques for nerve impregnation are notoriously capricious when applied to such organs, and so far the results have been indifferent. Cajal's, Bodian's, Romanes' and Pearson's techniques have been tried, and Agduhr's, Wrete's and Howarth and Samuel's methods are now being used.

Other tissues are less fickle in their behaviour towards metallic salts, and the results obtained with reticular fibres show that the idea of combining microradiographic and metallic impregnation techniques has definite possibilities. As the sections used are much thicker (40–400 μ) than those employed in histology, bundles of fibres may be traced over greater distances, especially if stereoscopic views are taken. If one desires to study the pattern rather than histological detail, e.g. the arrangement of reticular or nerve bundles in various structures, this method could provide an alternative to the drudgery of serial sections and wax reconstructions. The results obtained with reticulum suggest that similar results could be obtained with nerve bundles if the metallic impregnation methods for nerves were as uniform and reliable as those for reticular tissue.

The post-natal development of the inferior dental nerve of the cat. By A. Mohiuddin. *Department of Anatomy, St Mary's Hospital Medical School, London, W.* 2

The inferior dental nerve was examined in thirty-two cats of different ages between birth and maturity with a view to determining the effect, if any, of the loss of the milk teeth on the number of axons, myelin sheaths and the calibre spectrum of the nerve.

The adult nerves (five specimens) were found to contain an average of 5679 axons ($\sigma = 838$) and though the average for immature nerves (nine specimens) was smaller, 4730 ($\sigma = 231$) the difference is probably accounted for by the greater ease of obtaining complete counts with the coarser fibres in the adult nerves. It is concluded that there is no significant change in the number of axons between birth and maturity.

Of these axons, 53 % were myelinated in the kitten at birth. The percentage increased rapidly to 70 at 10 days. The adult percentage of myelinated fibres (89) is reached before the end of the second month.

The calibre spectrum of the nerve at birth showed a distribution around a single mode at about 4 μ. A bimodal distribution is reached by the end of the third month; after the fifth month the nerve is histologically mature and contains fibres ranging from 2 to 18 μ in diameter distributed round two modes at about 4 and 10 μ respectively.

The post-natal growth pattern of the inferior dental nerve appears to be very similar to that of some other peripheral nerves, and to show no peculiarities which can be associated with the loss of the milk teeth or the maturation of the permanent dentition.

Early changes in the shape and size of a nerve fibre after crushing. By G. CAUSEY and E. PALMER. *Department of Anatomy, University College, Gower Street, London, W.C.* 1

An investigation was made into the shape and size of the myelinated nerve fibres of the nerve to the medial head of the gastrocnemius of rabbits after crushing with forceps. The nerves were fixed *in situ* with a modified Flemming's solution and the differences in nerves fixed *in situ* and on cards is discussed. Measurements were made of the fibre diameters at various times after the crush, above and below the crushed region.

When fixation was begun by pouring the fixing fluid round the nerve with the crushing forceps still applied, there was found to be an increase in the fibre size in the regions 1 mm. above and below the site of disruption of the fibres, as compared with the uncrushed nerve of the opposite limb. Five hours after crushing, the mean fibre diameter within 1 mm. above and below the crush was larger than the control from the opposite side. At times between 5 and 48 hr. the mean diameter within 1 mm. above the crush was greater than the mean diameter within 1 mm. below the crush.

After 48 hr. the distal fibres began to vary greatly in diameter as they took on an unduloid form before the break-up of the internodal myelin. This effect was shown in graphically reconstructed individual fibres from serial sections. In these reconstructions the swelling of the end of the proximal axon was also seen in the first $\frac{1}{4}$–$\frac{1}{2}$ mm. above the disruption. The first node of Ranvier above and below showed retraction of the myelin at an early stage.

Sources and arrangement of the ganglionic arteries to the superior and middle cervical sympathetic ganglia. By E. L. PATTERSON. *Department of Anatomy, The University, Manchester* 13

There is a lack of detailed information concerning the constancy or otherwise of the sources of blood supply to the cervical sympathetic trunk and also in regard to the arrangement of the ganglionic arteries. These matters have been studied in twenty-five dissections of the new-born after arterial injection. The following observations were made:

(1) The superior cervical ganglion receives its blood supply from several sources: (*a*) the ascending pharyngeal artery, the principal and only constant source. The common arrangement of its ganglionic branches was described; (*b*) the superior thyroid artery, a very common source, though the extent of its contribution is somewhat variable; and (*c*) the inferior thyroid artery, which usually provides ganglionic branches either through its ascending cervical branch or by a longitudinal vessel ascending on the sympathetic trunk. Less commonly the occipital and internal carotid arteries provide a supplementary blood supply.

(2) The middle cervical ganglion shows variations in the pattern of its ganglionic vessels according to its relation to the loop of the inferior thyroid artery, its principal and commonly sole source of blood supply. The usual arrangement of these vessels in high and low positions of the ganglion was described. In the low position the thyro-cervical and costo-cervical trunks may contribute to its arterial supply.

(3) The ganglion intermédiaire receives branches similar in source and arrangement to those of a low middle cervical ganglion.

A brief note upon the trapezius muscle—persisting fallacies. By R. D. LOCKHART. *Department of Anatomy, The University, Aberdeen*

A short film and lantern slides were shown demonstrating the surface anatomy of the scapula and the trapezius muscle during movements of the arm.

From recent statements it is evident that the movements of the scapula upon the chest wall during elevation of the arm to the horizontal are not yet fully appreciated and that confusion also exists regarding the exact disposition of the lower fibres of the trapezius muscle.

The metabolism of cell division and cell differentiation. By R. J. O'Connor. *The John Burford Carlill Pathological Laboratories, Westminster School of Medicine, Horseferry Road, London, S.W.* 1

In the midbrain of the chicken embryo, from the third to the eighth day of incubation, the following have been measured:

(*a*) The number of dividing cells per cu.mm. tissue (*M*).

(*b*) The degree of differentiation expressed as the proportion of the marginal layer (*D*).

(*c*) The rate of oxygen consumption expressed as cu.mm. oxygen consumed per cu.mm. of tissue per hour (*R*).

(*d*) The rate of aerobic glycolysis measured by the carbon dioxide liberated from sodium bicarbonate by the acid produced, and expressed as cu.mm. carbon dioxide per cu.mm. of tissue per hour (*G*).

During the developmental period considered *R* remains constant but *M*, *D* and *G* undergo variations which can be expressed as,

$$M = aG - b, \quad \log D = p - qG,$$

where *a*, *b*, *p*, and *q* are constants that can be derived from the results obtained. The implications of these relationships are discussed.

APRIL 1950

An ordinary meeting of the Society, for the Session 1949–50, was held on Friday, 28 April 1950, in the Department of Anatomy, King's College, Strand, London, W.C. 2. Prof. J. Kirk (Vice-President) was in the Chair.

The following are the authors' abstracts of the papers read:

The formation and closure of the cervical sinus in the sheep. By R. J. Scothorne. *Department of Anatomy, The School of Medicine, Leeds*

In the sheep embryo the cervical sinus appears as a triangular depression bounded in front by the second arch, above and behind by the caudal limiting ridge (Frazer) and below by the epipericardial ridge. In its floor there lie the third and fourth arches and the second, third and fourth external pharyngeal grooves. The upper end of the second external groove is cut off as a cervical vesicle II which disappears without trace at about the 14 mm. stage of development. A second vesicle is formed from ectoderm of the caudal end of the cervical sinus by the submergence of both arches III and IV. This vesicle is intimately fused with the nodose ganglion of the vagus and with the third endodermal pouch. Prenant (1894) thought this vesicle to be endodermal in origin. Moreover, he ascribed to it a major role in the development of the head of the thymus. Verdun (1898), while correctly describing the cervical vesicle as derived from ectoderm, maintained that it did not persist after the 18 mm. stage. The present study confirms and extends the observations of Tordoir (1935), who showed that the cervical vesicle is still recognizable in embryos of 50 and 60 mm., attached to or embedded in the epithelium of the rostral end of the third pouch. Tordoir denied the cervical vesicle any part in the formation of the head of the thymus. While the question cannot be definitely answered by the present studies, there are theoretical and comparative grounds for believing that the head of the thymus in the sheep may be in part ectodermal in origin.

The histochemical identification of higher polysaccharides. By G. B. S. Roberts, and W. F. H. Jarrett. *Department of Pathology, The University, Glasgow, W.* 2

The introduction of the periodic acid Schiff reaction made it possible to stain all histological structures which contain higher polysaccharides (1:2 glycol grouping in a hexose chain).

The technique as originally employed was unable to differentiate the various groups of these substances. The use of the metachromatic reaction and of enzymatic hydrolysis makes it possible to identify four main chemical groups: glycogen, acid mucopolysaccharides, mucoproteins (including glycoproteins), and glycolipides.

A simplified scheme of identification by these methods is suggested, and some of the commoner fallacies discussed and methods for their recognition and elimination suggested.

Mitotic inhibition in embryonic tissue. By R. J. O'Connor. *The John Burford Carlill Pathological Laboratories, Westminster School of Medicine, Horseferry Road, London, S.W.* 1

Many investigators have measured mitotic inhibition by observations on tissue cultures. In the present experiments a different method has been used, namely, to expose fragments of the 6-day embryonic chick brain to various concentrations of the inhibitors in a saline medium for 90 min. Adequate concentrations produced a pycnotic fusion of chromosomes which could readily be detected in 'squash' preparations. Concentrations producing this change have been compared with concentrations producing inhibition of growth in tissue culture and no significant difference found.

When pycnotic fusion of chromosomes was produced in the embryonic brain tissue the associated effect on aerobic glycolysis was measured by the Cartesian diver micromanometer. Urethane, nitrogen mustard, sodium iodoacetate and sodium fluoride produced 50–70% inhibition, sodium azide increased aerobic glycolysis four to five times, while acridine compounds produced no alteration. Thus, with the exception of acridine compounds, pycnotic fusion of chromosomes is associated with an alteration of aerobic glycolysis. In the case of acridines it is suggested that these compounds interfered with the transfer of energy derived from aerobic glycolysis to that derived from some process essential to chromosome integrity. It is thus possible, in all compounds, to associate the pycnotic fusion of chromosomes with effects on aerobic glycolysis.

The foetal membranes of the Weddell seal (*Leptonychotes weddelli*). By D. V. Davies. *Department of Anatomy, St Thomas's Hospital, London, S.E.* 1

Observations on the arrangement of the foetal membranes of the seal are few and confined to the late stages. The material examined in the present investigation consists of eight uteri containing embryos ranging from under 6 to 180 mm. G.L., together with two non-pregnant uteri. In the earliest stage there is marked enlargement and activity of the uterine glands and the formation of crypts at the surface of the endometrium comparable to those described by Duval in the dog. By 6 mm. villosities from the allanto-chorion are penetrating the endometrium, the superficial parts of the glands have become occluded by hypertrophy and degeneration of their epithelium, whilst their deeper parts have become further dilated. By 18 mm. the zonary epithelio-chorial placenta is established; the deeper portions of the uterine glands persist throughout the series. Contrary to previous accounts, in the seal there are peripheral brown borders to the placenta together with an irregular haematoma connecting them mesometrially. The yolk sac is intimately related to this haematoma and possibly retains to term its connexion with the chorion in this region.

The umbilical cord is typically a short flattened structure containing at the 180 mm. stage two umbilical arteries, the allantoic stalk, one umbilical vein and two vitelline vessels. There is at this stage no trace of an obliterated umbilical vein or vitello-intestinal stalk. On reaching the peritoneal cavity the vitelline vessels traverse this independently and unsupported by mesenteries, to join the supérior mesenteric artery and portal vein respectively.

The autonomic and somatic innervation of the feline articular capsule. By E. P. Samuel. *Department of Anatomy, The University, Manchester* 13

A study of the knee joints of eighteen cats subjected to uni- or bi-lateral lumbosacral sympathectomy combined with a femoral periarticular sympathectomy shows that the large nerve bundles which ramify and divide, forming a coarse network in the fibrous capsular

ligament, consist of autonomic and somatic fibres. As these bundles migrate from vessel to vessel they distribute their autonomic constituents to the blood vessels, gradually shedding most of their somatic fibres into the surrounding tissue. Thus, when the vascular synovial tissue is reached the nerves running with the blood vessels are mainly autonomic. From the somatic fibres in the nerve bundles and those running with the blood vessels a non-medullated nerve plexus is formed in the inner layers of the fibrous capsular ligament. Other individual somatic fibres terminate in various types of nerve endings. The autonomic fibres, on the other hand, form a plexus between the tunica media and adventitia or are embedded in the media of the vessels, and compose the majority of the fibres accompanying the arterioles and capillaries in the junctional tissue and synovial membrane; they end as fine threads or beaded endings. Nevertheless, the persistence of some nerve fibres in the synovial membrane of sympathectomized animals does demonstrate that the membrane receives a definite somatic nerve supply.

An oblique proximal septum of the pulp space. By J. L. Wilkinson.
Department of Anatomy, The University, Manchester 13

Despite its considerable clinical significance, the description of pulp-space anatomy lacks precision and detail. In view of pulp-space localization of pus, it was decided to re-investigate this space. So far, sixty fingers and several toes have been examined.

It is difficult to demonstrate the precise nature and attachments of the pulp-space fascia by ordinary dissection, so 100 μ coronal, sagittal and oblique sections of decalcified fingers were prepared and examined. They were stained by Verhoeff, Mallory and Van Gieson techniques. These sections, apart from illustrating the general arrangement of pulp-space fascia, reveal a definite oblique septum proximal to the pulp space. Ventrally it extends from the deep fascia opposite the distal volar crease to the fibrous and synovial tendon sheaths, and laterally it extends from these sheaths and the joint capsule to the deep fascia and Cleland's ligaments; the fibrous tendon sheath is a tenuous structure at its distal extremity. The septum described consists mainly of collagen bundles, with little elastic tissue. The central fibres decussate, but those situated more laterally maintain the side of the finger from which they originate—a disposition reminiscent of the arrangement of nerve fibres in the optic chiasma. The septum is pierced on each side by the neurovascular bundle. A large midline subcutaneous vein is commonly present, for at this level venae commitantes are usually very small or absent.

Examination of the region of the middle volar crease also revealed a definite arrangement of connective tissue between tendon sheath and skin, but no true septum.

Twenty pulp spaces were injected, under controlled pressure and temperature, with gelatine carmine or colloidal silver iodine, and in the latter fluoroscopic examination was followed by serial radiographs. It was found that the oblique proximal septum of the pulp space will withstand pressures up to 150 mm. Hg.

Colloidal silver iodine was also introduced into the synovial sheath by centrifugal force, and the relationships between the sheath, septum and pulp space were studied.

The upper limits of the pressure of pus in pulp-space infections was determined in a few patients, and correlated with the above experiments. The clinical significance of these findings will be briefly discussed.

INDEX TO VOLUME 84

Abbie, A. A. Closure of cranial articulations in the skull of the Australian aborigine, 1

Abdominal wall muscles, anterior, in man, electromyographic study of patterns of activity of the. By W. F. Floyd and P. H. S. Silver, 132

Adrenal cortex of the albino rat, cytological and histochemical variations in the. By A. J. Cain and R. G. Harrison, 196

Aitken, J. T. Growth of nerve implants in voluntary muscle, 38

Allison, A. C. Book review. Ouvrages sur l'Organe Voméro-Nasal. By Louis Jacobson, 324

Anastomoses, arterial, a method for the study of. By D. A. McDonald and J. M. Potter, 327

Armstrong, J. A. An experimental study of the visual pathways in a reptile (*Lacerta vivipara*), 146

Australian aborigine, closure of cranial articulations in the skull of the. By A. A. Abbie, 1

Azygos veins in the albino rat, the development of the. By H. Butler, 83

Balinsky, B. I. On the prenatal growth of the mammary gland rudiment in the mouse, 227

Billingham, R. E. and Medawar, P. B. A note on the specificity of the corneal epithelium, 50

Books received, 325

Book Reviews:

Angewandte und Topographische Anatomie. Ein Lehrbuch für Studierende und Ärzte. By Gian Töndury. Reviewed by J. D. Boyd, 399

A Cyto-architectonic Atlas of the Rhombencephalon of the Rabbit. By H. Meessen and J. Olszewski. Reviewed by W. E. Le Gros Clark, 57

Functional Localization in the Frontal Lobes and Cerebellum. By J. F. Fulton. Reviewed by W. E. Le Gros Clark, 57

History of the Primates. By W. E. Le Gros Clark. Reviewed by C. M. West, 324

Man the Toolmaker. By Kenneth P. Oakley. Reviewed by C. M. West, 324

Ouvrages sur l'Organe Voméro-Nasal. By Louis Jacobson. Reviewed by A. C. Allison, 324

The Temporal Bone and the Ear. By Theodore H. Bast and Barry J. Anson. Reviewed by C. M. West, 57

Textbook of Histology. By J. F. Nonidez and W. F. Windle. Reviewed by K. C. Richardson, 58

The Vertebrate Body. By Alfred Sherwood Romer. Reviewed by G. R. de Beer, 399

Borghese, E. The development *in vitro* of the submandibular and sublingual glands of *Mus musculus*, 287

— Explantation experiments on the influence of the connective tissue capsule on the development of the epithelial part of the submandibular gland of *Mus musculus*, 303

Boyd, J. D. Book review. Angewandte und Topographische Anatomie. Ein Lehrbuch für Studierende und Ärzte. By Gian Töndury, 399

Butler, H. The development of the azygos veins in the albino rat, 83

Cain, A. J. and Harrison, R. G. Cytological and histochemical variations in the adrenal cortex of the albino rat, 196

Cervical sympathetic ganglia, sources of arterial blood supply to the superior and middle, and the ganglion intermédiaire. By E. L. Patterson, 329

Clark, W. E. Le Gros. Book review. A Cyto-architectonic Atlas of the Rhombencephalon of the Rabbit. By H. Meessen and J. Olszewski, 57

— Book review. Functional Localization in the Frontal Lobes and Cerebellum. By J. F. Fulton, 57

Closure of cranial articulations in the skull of the Australian aborigine. By A. A. Abbie, 1

Comparison of fracture repair in the frog, lizard and rat. By J. J. Pritchard and A. J. Ruzicka, 236

Connective tissue capsule, explantation experiments on the influence of the, on the development of the epithelial part of the submandibular gland of *Mus musculus*. By E. Borghese, 303

Cornea, a critical evaluation of methods used to demonstrate tissue neural elements, illustrated by reference to the. By G. Weddell and E. Zander, 168

Corneal epithelium, a note on the specificity of the. By R. E. Billingham and P. B. Medawar, 50

Coronary arteries in Dipnoan fishes, a description of the, and some remarks on their importance from the evolutionary standpoint. By G. E. H. Foxon, 121

Critical evaluation of methods used to demonstrate tissue neural elements, illustrated by reference to the cornea. By G. Weddell and E. Zander, 168

Cytological and histochemical variations in the adrenal cortex of the albino rat. By A. J. Cain and R. G. Harrison, 196

Davies, J. The pronephros and the early development of the mesonephros in the duck, 95

De Beer, G. R. Book review. The Vertebrate Body. By Alfred Sherwood Romer, 399

Description of the coronary arteries in Dipnoan fishes and some remarks on their importance from the evolutionary standpoint. By G. E. H. Foxon, 121

Development of the azygos veins in the albino rat. By H. Butler, 83

Development *in vitro* of the submandibular and sublingual glands of *Mus musculus*. By E. Borghese, 287

Differentiation of mammalian epidermis in tissue culture. By Jean Hanson, 30

Doran, F. S. A. The intra-mural blood supply of the upper jejunum in man, 283

Duck, the pronephros and the early development of the mesonephros in the. By J. Davies, 95

Effect of malnutrition as compared with hypophysectomy on organ weight of the albino rat. By L. M. Rinaldini, 262

Electromyographic study of patterns of activity of the anterior abdominal wall muscles in man. By W. F. Floyd and P. H. S. Silver, 132
Epidermis, mammalian, differentiation of, in tissue culture. By Jean Hanson, 30
Epithelium, corneal, a note on the specificity of the. By R. E. Billingham and P. B. Medawar, 50
Experimental study of the visual pathways in a reptile (*Lacerta vivipara*). By J. A. Armstrong, 146
Explantation experiments on the influence of the connective tissue capsule on the development of the epithelial part of the submandibular gland of *Mus musculus*. By E. Borghese, 303

Fate of the nerves of the deciduous teeth. By A. Mohiuddin, 319
Fisher, R. B. and Parsons, D. S. The gradient of mucosal surface area in the small intestine of the rat, 272
Fishes, Dipnoan, a description of the coronary arteries in, and some remarks on their importance from the evolutionary standpoint, 121
Flexor muscles of the forearm and hand in lizards and mammals. By R. Wheeler Haines, 13
Floyd, W. F. and Silver, P. H. S. Electromyographic study of patterns of activity of the anterior abdominal wall muscles in man, 132
Forearm and hand in lizards and mammals, the flexor muscles of the. By R. Wheeler Haines, 13
Foxon, G. E. H. A description of the coronary arteries in Dipnoan fishes and some remarks on their importance from the evolutionary standpoint, 121
Fracture repair in the frog, lizard and rat, comparison of. By J. J. Pritchard and A. J. Ruzicka, 236
Frog, lizard and rat, comparison of fracture repair in the. By J. J. Pritchard and A. J. Ruzicka, 236

Ganglia, sources of arterial blood supply to the superior and middle cervical sympathetic, and the ganglion intermédiaire. By E. L. Patterson, 329
Gland, mammary, rudiment, on the prenatal growth of the, in the mouse. By B. I. Balinsky, 227
Gland, submandibular, of *Mus musculus*, explantation experiments on the influence of the connective tissue capsule on the development of the epithelial part of the. By E. Borghese, 303
Glands, submandibular and sublingual, of *Mus musculus*, the development *in vitro* of the. By E. Borghese, 287
Gradient of mucosal surface area in the small intestine of the rat. By R. B. Fisher and D. S. Parsons, 272
Growth of nerve implants in voluntary muscle. By J. T. Aitken, 38

Haines, R. Wheeler. The flexor muscles of the forearm and hand in lizards and mammals, 13
Hanson, Jean. Differentiation of mammalian epidermis in tissue culture, 30
Harrison, R. G. *See* Cain, A. J., joint authors, 196
Histochemical changes produced by cold and adrenaline in the suprarenal cortex of the adult male rat. By Frances B. Robinson and J. M. Yoffey, 32
Hypophysectomy, effect of malnutrition as compared with, on organ weight of the albino rat. By L. M. Rinaldini, 262

Intestine, small, of the rat, the gradient of mucosal surface area in the. By R. B. Fisher and D. S. Parsons, 272
Intra-mural blood supply of the upper jejunum in man. By F. S. A. Doran, 283

Jejunum, upper, in man, the intra-mural blood supply of the. By F. S. A. Doran, 283

Lizard, frog and rat, comparison of fracture repair in the. By J. J. Pritchard and A. J. Ruzicka, 236

McDonald, D. A. and Potter, J. M. A method for the study of arterial anastomoses, 327
Malnutrition, effect of, as compared with hypophysectomy on organ weight of the albino rat. By L. M. Rinaldini, 262
Mammary gland rudiment in the mouse, on the prenatal growth of the. By B. I. Balinsky, 227
Mandible, human, the relation of the direction of the mental foramen to the growth of the. By R. Warwick, 116
Medawar, P. B. *See* Billingham, R. E., joint authors, 50
Mental foramen, the relation of the direction of the, to the growth of the human mandible. By R. Warwick, 116
Mesonephros, the pronephros and the early development of the, in the duck. By J. Davies, 95
Method for the study of arterial anastomoses. By D. A. McDonald and J. M. Potter, 327
Mohiuddin, A. The fate of the nerves of the deciduous teeth, 319
Mouse, on the prenatal growth of the mammary gland rudiment in the. By B. I. Balinsky, 227
Mus musculus, the development *in vitro* of the submandibular and sublingual glands of. By E. Borghese, 287
Mus musculus, explantation experiments on the influence of the connective tissue capsule on the development of the epithelial part of the submandibular gland of. By E. Borghese, 303
Muscle, voluntary, growth of nerve implants in. By J. T. Aitken, 38
Muscles, anterior abdominal wall, in man, electromyographic study of patterns of activity of the. By W. F. Floyd and P. H. S. Silver, 132
Muscles, the flexor, of the forearm and hand, in lizards and mammals. By R. Wheeler Haines, 13

Nerve fibres, the staining of, in paraffin sections with silver. By G. J. Romanes, 104
Nerve implants, growth of, in voluntary muscle. By J. T. Aitken, 38
Nerves of the deciduous teeth, the fate of the. By A. Mohiuddin, 319
Nerves, human, the relationship between internodal length and growth in. By A. D. Vizoso, 342
Note on the specificity of the corneal epithelium. By R. E. Billingham and P. B. Medawar, 50

Parsons, D. S. *See* Fisher, R. B., joint authors, 272
Patterson, E. L. Sources of arterial blood supply to the superior and middle cervical sympathetic ganglia and the ganglion intermédiaire, 329

Potter, J. M. *See* McDonald, D. A., joint authors, 327
Prenatal growth of the mammary gland rudiment in the mouse. By B. I. Balinsky, 227
Pritchard, J. J. and Ruzicka, A. J. Comparison of fracture repair in the frog, lizard and rat, 236
Proceedings of the Anatomical Society of Great Britain and Ireland. (*See* supplementary index), 60
Pronephros and the early development of the mesonephros in the duck. By J. Davies, 95

Rat, albino. Cytological and histochemical variations in the adrenal cortex of the. By A. J. Cain and R. G. Harrison, 196
Rat, albino, the development of the azygos veins in the. By H. Butler, 83
Rat, albino, effect of malnutrition as compared with hypophysectomy on organ weight of the. By L. M. Rinaldini, 262
Rat, frog, lizard and, comparison of fracture repair in the. By J. J. Pritchard and A. J. Ruzicka, 236
Relation of the direction of the mental foramen to the growth of the human mandible. By R. Warwick, 116
Relationship between internodal length and growth in human nerves. By A. D. Vizoso, 342
Reptile (*Lacerta vivipara*), an experimental study of the visual pathways in a. By J. A. Armstrong, 146
Richardson, K. C. Book review. Textbook of Histology. By J. F. Nonidez and W. F. Windle, 58
Rinaldini, L. M. Effect of malnutrition as compared with hypophysectomy on organ weight of the albino rat, 262
Robinson, Frances B. and Yoffey, J. M. Histochemical changes produced by cold and adrenaline in the suprarenal cortex of the adult male rat, 32
Romanes, G. J. The staining of nerve fibres in paraffin sections with silver, 104
Ruzicka, A. J. *See* Pritchard, J. J., joint authors, 236

Silver, P. H. S. *See* Floyd, W. F., joint authors, 132
Skull of the Australian aborigine, closure of cranial articulations in the. By A. A. Abbie, 1
Sources of arterial blood supply to the superior and middle cervical sympathetic ganglia and the ganglion intermédiaire. By E. L. Patterson, 329
Staining of nerve fibres in paraffin sections with silver. By G. J. Romanes, 104
Suprarenal cortex of the adult male rat, histochemical changes produced by cold and adrenaline in the. By Frances B. Robinson and J. M. Yoffey, 32

Teeth, the fate of the nerves of the deciduous. By A. Mohiuddin, 319
Tissue culture, differentiation of mammalian epidermis in. By Jean Hanson, 30
Tissue neural elements, a critical evaluation of methods used to demonstrate, illustrated by reference to the cornea. By G. Weddell and E. Zander, 168

Veins, azygos, the development of the, in the albino rat. By H. Butler, 83
Visual pathways in a reptile (*Lacerta vivipara*), an experimental study of the. By J. A. Armstrong, 146
Vizoso, A. D. The relationship between internodal length and growth in human nerves, 342

Warwick, R. The relation of the direction of the mental foramen to the growth of the human mandible, 116
Weddell, G. and Zander, E. A critical evaluation of methods used to demonstrate tissue neural elements, illustrated by reference to the cornea, 168
West, C. M. Book review. The Temporal Bone and the Ear. By Theodore H. Bast and Barry J. Anson, 57
— Book reviews. History of the Primates (an introduction to the study of fossil man) by W. E. Le Gros Clark, and Man the Toolmaker by Kenneth P. Oakley, 324

Yoffey, J. M. *See* Robinson, Frances B., joint authors, 32

Zander, E. *See* Weddell, G., joint authors, 168

SUPPLEMENTARY INDEX OF PROCEEDINGS

Abdominal wall, anterior, in man, electromyographic investigation of muscle movement patterns studied with surface electrodes on the. By W. F. Floyd and P. H. S. Silver, 75
Aitken, J. T. Growth of nerve implants in muscle, 65
Alimentary tract, upper, and the menstrual cycle, squamous cells of the. By I. D. Henderson, 404
Allison, A. C. and Meyer, M. Secondary olfactory connexions in monkeys, 63
Amoroso, E. C. The source and composition of the uterine milk in the mare, 78
Amoroso, E. C. and Murray, C. B. Implantation and the yolk-sac placenta in the cat, 71
Anastomoses, arterio-venous, in the human stomach. By T. E. Barlow, 405
Armstrong, J. A. An experimental study of optic pathways in *Lacerta vivipara*, 70
Articular capsule, feline, the autonomic and somatic innervation of the. By E. P. Samuel, 408
Ashton, E. H. *See* Zuckerman, S., joint authors, 401
Atrio-ventricular bundle, the nervous constituent of the. By E. J. Field, 65

Bacsich, P. Some observations on the human foetal ovary, 75
Bailey, R. A. *See* Glees, P., joint authors, 64
Balinsky, B. I. On the pre-natal growth of the mammary gland rudiment, 67
Barlow, T. E. Arterio-venous anastomoses in the human stomach, 405
Baxter, J. S. Alkaline phosphatase in the mesonephric and metanephric tubules of man, 77
Bentall, H. H. *See* Harrison, R. J., joint authors, 400
Bones, long, of the limbs, the nutrient canal in the. By H. Hughes, 68
Bourne, G. H. Collagen and pre-collagen, 61
Bowden, R., Napier, J. and Whillis, J. Movements of digits in ulnar paralysis. (Film), 76
Boyd, J. D. Afferent nerve supply of innominate-subclavian arch in the human embryo and foetus, 67
— Argentaffin dendritic cells in hair follicles, 62
— 'Intermediate' lumbar sympathetic ganglia in the human foetus, 401
— Müller's (spheno-maxillary) muscle in the human embryo and foetus, 72
— Nerve supply of the tonsil, 79
Brain and cord, structure of axones within. By M. A. MacConaill, 69
Breward, Madge M. and Zuckerman, S. Multiple ovarian grafts, 68
Brierley, J. B. and Field, E. J. The fate of an intraneural crystalloid injection as demonstrated by the radio-active tracer technique, 61

Cathcart, E. P., Gairns, F. W. and Garven, H. S. D. The innervation of the human quiescent nipple, 67
Causey, G. The effect of pressure on conduction and nerve fibre size, 65
Causey, G. and Palmer, E. Early changes in the shape and size of a nerve fibre after crushing, 406
Cell division and cell differentiation, the metabolism of. By R. J. O'Connor, 407
Cervical sinus in the sheep, the formation and closure of the. By R. J. Scothorne, 407
Chicken embryo, regional differences in the oxygen consumption of the. By R. J. O'Connor, 401
Collagen and pre-collagen. By G. H. Bourne, 61
Conceptus, a pre-somite human, of about 14 days of age, with special reference to the yolk-sac formation. By W. R. M. Morton, 66
Cornea, observations on the innervation of the. By G. Weddell and E. Zander, 79
Corneal nerves, living, neurological techniques in relation to the anatomy of. By E. Zander and G. Weddell, 78
Crawford, G. N. C. Experiments on the longitudinal growth of tendons in rabbits, 68

Davies, D. V. The foetal membranes of the Weddell seal (*Leptonychotes weddelli*), 408
Davies, J. The pronephros and mesonephros of the duck, 72
Dawson, B. H. The blood supply of the human hypothalamus, 80
Dendritic cells, argentaffin, in hair follicles. By J. D. Boyd, 62
Dental nerve, inferior, of the cat, the post-natal development of the. By A. Mohiuddin, 405
Doran, F. S. A. The intramural blood supply of the upper jejunum in man. A radiological study, 403
Durward, A. and Rudall, K. M. Experiments on hair growth in the rat, 66

Ear, methods employed in demonstrating the anatomy of the. By Oliver Gray, 401
Embryo, chicken, regional differences in the oxygen consumption of the. By R. J. O'Connor, 401
Embryo, congenital cystic eye in a 5 mm. human. By W. R. M. Morton, 400
Embryo and foetus, human, afferent nerve supply of innominate-subclavian arch in the. By J. D. Boyd, 67
Embryo and foetus, human, Müller's (spheno-maxillary) muscle in the. By J. D. Boyd, 72
Embryonic tissue, mitotic inhibition in. By R. J. O'Connor, 408
Epiglottis, the neural pattern of the. By W. H. Feindel, 64
Evans, D. H. L. An investigation of the distribution of medullated fibres in the vagus nerve of the rabbit, 73
Eye, congenital cystic, in a 5 mm. human embryo. By W. R. M. Morton, 400

Feindel, W. H. The neural pattern of the epiglottis, 64
Fernand, V. S. V. and Young, J. Z. The composition of muscle nerves, 402
Field, E. J. The nervous constituent of the atrio-ventricular bundle, 65
— *See* Brierley, J. B., joint authors, 61
Field, E. J., Grayson, J. and Rogers, A. F. The effect of activity on the blood flow in the spinal cord, 402

Floyd, W. F. and Silver, P. H. S. Electromyographic investigation of muscle movement patterns studied with surface electrodes on the anterior abdominal wall in man, 75
Foetus, human, 'intermediate' sympathetic ganglia in the. By J. D. Boyd, 401
Fracture repair in *Lacerta vivipara*, an experimental study of. By J. J. Pritchard and A. J. Ruzicka, 69
Franklin, K. J. The renal shunt mechanism and anoxia (Preliminary note), 63

Gairns, F. W. *See* Cathcart, E. P., joint authors, 67
Ganglia, 'intermediate' lumbar sympathetic, in the human foetus. By J. D. Boyd, 401
Ganglia superior and middle cervical sympathetic, sources and arrangement of the ganglionic arteries to the. By E. L. Patterson, 406
Garven, H. S. D. *See* Cathcart, E. P., joint authors, 67
Gland, mammary, the development of the, (early intra-uterine phase). By E. S. R. Hughes, 60
Gland rudiment, mammary, on the pre-natal growth of the. By B. I. Balinsky, 67
Glees, P., Oppenheimer, D. R. and Bailey, R. A. Observations on ascending and descending tract degeneration following midbrain tractotomy in the human, 64
Goldby, F. and Mohiuddin, A. Preganglionic fibres of the vagus, 79
Gray, Oliver. Methods employed in demonstrating the anatomy of the ear, 401
Grayson, J. *See* Field, E. J., joint authors, 402

Hair follicles, argentaffin dendritic cells in. By J. D. Boyd, 62
Hair growth in the rat, experiments on. By A. Durward and K. M. Rudall, 66
Hairs, vibrissa-like, in the rabbit's leg, the innervation of. By G. Weddell, 69
Harris, G. W. Experiments on the hypophysial portal vessels, 62
Harris, H. and Joseph, J. Extension at the metacarpo-phalangeal and interphalangeal joints of the thumb, 73
Harrison, R. J. Observations on the seal ovary, 400
Harrison, R. J. and Bentall, H. H. The inferior vena cava in the seal, 400
Henderson, I. D. Squamous cells of the upper alimentary tract and the menstrual cycle, 404
Hill, W. C. Osman. Osteological racial differences in the newborn, 73
Howarth, F. Observations on the departure of substances from the spinal theca, 80
Hughes, E. S. R. The development of the mammary gland (early intra-uterine phase), 60
Hughes, H. The nutrient canal in the long bones of the limbs, 68
Hutchison, J. and Wyburn, G. M. Regenerated sensory pattern in grafted skin, 67
Hypophysial portal vessels, experiments on the. By G. W. Harris, 62
Hypothalamus, human, the blood supply of the. By B. H. Dawson, 80

Implantation and the yolk-sac placenta in the cat. By E. C. Amoroso and C. B. Murray, 71
Injection, the fate of an intra-neural crystalloid, as demonstrated by the radio-active tracer technique. By J. B. Brierley and E. J. Field, 61
Innominate-subclavian arch in the human embryo and foetus, afferent nerve supply of. By J. D. Boyd, 67
Intervertebral disc, the reaction of the, to experimental lesions. By R. Walmsley and J. W. Smith, 74
Intestine, large, alkaline phosphatase in the. By B. F. Martin, 404

Jacoby, F. *See* Martin, B. F., joint authors, 61
Jarrett, W. F. H. *See* Roberts, G. B. S., joint authors, 407
Jaw, limbs and, in man, the relative sizes of. By R. H. Shepherd, D. A. Sholl and A. Vizoso, 60
Jaw movements in foetal sheep, the anatomy of. By J. H. Scott, 77
Jejunum, upper, in man, the intramural blood supply of the. A radiological study. By F. S. A. Doran, 403
Joint sensation, an histological and sensory investigation to determine the role of the articular capsule in. By E. P. Samuel, 77
Joints, subtalar and calcaneo-cuboid, limitation of movement at the. By J. W. Smith, 75
Joints of the thumb, extension at the metacarpo-phalangeal and interphalangeal. By H. Harris and J. Joseph, 73
Joseph, J. *See* Harris, H., joint authors, 73

Kidney, the nerve supply of the. By G. A. G. Mitchell, 70
Kirk, J. A note on pigmentation of the skin, 75

Lacrimal fluid, the distribution of the. By E. Wolff, 60
Larynx, action of posterior crico-arytenoid muscle of the. By V. E. Negus, 68
Last, R. J. The popliteus muscle and the lateral meniscus, 62
Limbs and jaw in man, the relative sizes of. By R. H. Shepherd, D. A. Sholl and A. D. Vizoso, 60
Limbs, the nutrient canal in the long bones of the. By H. Hughes, 68
Lockhart, R. D. A brief note upon the trapezius muscle-persisting fallacies, 406
Lumbar sympathetic ganglia, 'intermediate', in the human foetus. By J. D. Boyd, 401
Lungs, the blood supply to the. By E. W. Walls, 81

MacConaill, M. A. Structure of axones within brain and cord, 69
McGregor, I. The return of sweating in grafted skin, 76
Martin, B. F. Alkaline phosphatase in the large intestine, 404
Martin, B. F. and Jacoby, F. Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase, 61
Menstrual cycle, squamous cells of the upper alimentary tract and the. By I. D. Henderson, 404
Meyer, M. *See* Allison, A. C., joint authors, 63
Midbrain tractotomy in the human, observations on ascending and descending tract degeneration following. By P. Glees, D. R. Oppenheimer and R. A. Bailey, 64

Mitchell, G. A. G. Microradiographic demonstration of tissues treated by metallic impregnation, 405
— The nerve supply of the kidney, 70
— The renal fascia, 76
Mitchell, R. Some observations on the histology of the infant testicle, 75
Mohiuddin, A. *See* Goldby, F., joint authors, 79
Monkey, green, the influence of geographical isolation on the teeth of the. By S. Zuckerman and E. H. Ashton, 401
Monkey, rhesus, a study of retrograde degeneration in the oculomotor nucleus of the, with a note on a method of recording it. By R. Warwick, 402
Monro, P. A. G. Sudomotor escape areas after thoraco-lumbar sympathectomies, 401
Morton, W. R. M. Congenital cystic eye in a 5 mm. human embryo, 400
— A pre-somite human conceptus of about 14 days of age, with special reference to the yolk-sac formation, 66
Murray, C. B. *See* Amoroso, E. C., joint authors, 71
Muscle, crico-arytenoid, of the larynx, action of posterior. By V. E. Negus, 68
Muscle, growth of nerve implants in. By J. T. Aitken, 65
Muscle movement patterns, electromyographic investigation of, studied with surface electrodes on the anterior abdominal wall in man. By W. F. Floyd and P. H. S. Silver, 75
Muscle, Müller's (spheno-maxillary), in the human embryo and foetus. By J. D. Boyd, 72
Muscle nerves, the composition of. By V. S. V. Fernand and J. Z. Young, 402
Muscle-persisting fallacies, trapezius, a brief note upon the. By R. D. Lockhart, 406
Muscle, popliteus, and the lateral meniscus. By R. J. Last, 62

Napier, J. *See* Bowden, R., joint authors, 76
Negus, V. E. Action of posterior crico-arytenoid muscle of the larynx, 68
Nerve fibre, early changes in the shape and size of a, after crushing. By G. Causey and E. Palmer, 406
Nerve fibre size, the effect of pressure on conduction and. By G. Causey, 65
Nerve implants in muscle, growth of. By J. T. Aitken, 65
Nerve, inferior dental, of the cat, the post-natal development of the. By A. Mohiuddin, 405
Nerve, mental, growth changes in the, and their relationship to the direction of the mental foramen. By R. Warwick, 72
Nerve, vagus, of the rabbit, an investigation of the distribution of medullated fibres in the. By D. H. L. Evans, 73
Nerves, corneal, neurological techniques in relation to the anatomy of living. By E. Zander and G. Weddell, 78
Nerves, muscle, the composition of. By V. S. V. Fernand and J. Z. Young, 402
Nervous system, central, of *Natrix sipedon*, the myelinization of the. By Francis James Warner, 403
Nipple, human quiescent, the innervation of the. By E. P. Cathcart, F. W. Gairns and H. S. D. Garven, 67

O'Connor, R. J. The metabolism of cell division and cell differentiation, 407
— Mitotic inhibition in embryonic tissue, 408
— Regional differences in the oxygen consumption of the chicken embryo, 401
Oculomotor nucleus of the rhesus monkey, a study of retrograde degeneration in the, with a note on a method of recording it. By R. Warwick, 402
Olfactory connexions, secondary, in monkeys. By A. C. Allison and M. Meyer, 63
Oppenheimer, D. R. *See* Glees, P., joint authors, 64
Optic nerve in the mouse, medullation of the. By G. J. Romanes, 79
Optic pathways in *Lacerta vivipara*, an experimental study of. By J. A. Armstrong, 70
Osteoblast, maturation phenomena in the. By J. J. Pritchard, 404
Osteological racial differences in the newborn. By W. C. Osman Hill, 73
Ovarian grafts, multiple. By Madge M. Breward and S. Zuckerman, 68
Ovary, human foetal, some observations on the. By P. Bacsich, 75
Ovary, seal, observations on the. By R. J. Harrison, 400

Pain, the reference of, aroused in the skin. By D. C. Sinclair, 64
Palmer, E. *See* Causey, G., joint authors, 406
Patterson, E. L. Sources and arrangement of the ganglionic arteries to the superior and middle cervical sympathetic ganglia, 406
Phosphatase, alkaline, diffusion phenomenon complicating the histochemical reaction for. By B. F. Martin and F. Jacoby, 61
Phosphatase, alkaline, in the large intestine. By B. F. Martin, 404
Phosphatase, alkaline, in the mesonephric and metanephric tubules of man. By J. S. Baxter, 77
Placenta, yolk-sac, implantation and the, in the cat. By E. C. Amoroso and C. B. Murray, 71
Polysaccharides, higher, the histochemical identification of. By G. B. S. Roberts and W. F. H. Jarrett, 407
Pritchard, J. J. Maturation phenomena in the osteoblast, 404
Pritchard, J. J. and Ruzicka, A. J. An experimental study of fracture repair in *Lacerta vivipara*, 69
Pronephros and mesonephros of the duck. By J. Davies, 72
Pulp space, an oblique proximal septum of the. By J. L. Wilkinson, 409

Racial differences, osteological, in the newborn. By W. C. Osman Hill, 73
Radio-active tracer technique, the fate of an intra-neural crystalloid injection as demonstrated by the. By J. B. Brierley and E. J. Field, 61
Renal fascia. By G. A. G. Mitchell, 76
Renal shunt mechanism and anoxia (preliminary note). By K. J. Franklin, 63
Roberts, G. B. S. and Jarrett, W. F. H. The histochemical identification of higher polysaccharides, 407
Rogers, A. F. *See* Field, E. J., joint authors, 402
Romanes, G. J. Medullation of the optic nerve in the mouse, 79

Rudall, K. M. *See* Durward, A., joint authors, 66
Ruzicka, A. J. *See* Pritchard, J. J., joint authors, 69

Samuel, E. P. The autonomic and somatic innervation of the feline articular capsule, 408
— An histological and sensory investigation to determine the role of the articular capsule in joint sensation, 77
Scothorne, R. J. The formation and closure of the cervical sinus in the sheep, 407
Scott, J. H. The anatomy of jaw movements in foetal sheep, 77
Seal, the inferior vena cava in the. By R. J. Harrison and H. H. Bentall, 400
Seal ovary, observations on the. By R. J. Harrison, 400
Seal, Weddell, (*Leptonychotes weddelli*) the foetal membranes of the. By D. V. Davies, 408
Shepherd, R. H., Sholl, D. A. and Vizoso, A. The relative sizes of limbs and jaw in man, 60
Sholl, D. A. *See* Shepherd, R. H., joint authors, 60
Silver, P. H. S. *See* Floyd, W. F., joint authors, 75
Sinclair, D. C. The reference of pain aroused in the skin, 64
Skin, grafted, regenerated sensory pattern in. By J. Hutchison and G. M. Wyburn, 67
Skin, grafted, the return of sweating in. By I. McGregor, 76
Skin, pigmentation of the, a note on. By J. Kirk, 75
Skin, the reference of pain aroused in the. By D. C. Sinclair, 64
Smith, J. W. Limitation of movement at the subtalar and calcaneo-cuboid joints, 75
— *See* Walmsley, R., joint authors, 74
Spinal cord, the effect of activity on the blood flow in the. By E. J. Field, J. Grayson and A. F. Rogers, 402
Spinal theca, observations on the departure of substances from the. By F. Howarth, 80
Stomach, arterio-venous anastomoses in the human. By T. E. Barlow, 405
Sudomotor escape areas after thoraco-lumbar sympathectomies. By P. A. G. Monro, 401

Teeth, of the green monkey, the influence of geographical isolation on the. By S. Zuckerman and E. H. Ashton, 401
Tendons in rabbits, experiments on the longitudinal growth of. By G. N. C. Crawford, 68
Testicle, infant, some observations on the histology of the. By R. Mitchell, 75
Thoraco-lumbar sympathectomies, sudomotor escape areas after. By P. A. G. Monro, 401
Thrombosis, popliteal, the anatomy of. By F. R. Wilde, 74
Thumb, extension at the metacarpo-phalangeal and interphalangeal joints of the. By H. Harris and J. Joseph, 73
Tissue, embryonic, mitotic inhibition in. By R. J. O'Connor, 408
Tissues treated by metallic impregnation, microradiographic demonstration of. By G. A. G. Mitchell, 405
Tonsil, nerve supply of the. By J. D. Boyd, 79
Trapezius muscle-persisting fallacies, a brief note upon the. By R. D. Lockhart, 406

Ulnar paralysis, movements of digits in. (Film.) By R. Bowden, J. Napier and J. Whillis, 76
Uterine milk in the mare, the source and composition of the. By E. C. Amoroso, 78

Vagus nerve of the rabbit, an investigation of the distribution of medullated fibres in the. By D. H. L. Evans, 73
Vagus, preganglionic fibres of the. By F. Goldby and A. Mohiuddin, 79
Vena cava, inferior, in the seal. By R. J. Harrison and H. H. Bentall, 400
Vizoso, A. *See* Shepherd, R. H., joint authors, 60

Walls, E. W. The blood supply to the lungs, 81
Walmsley, R. and Smith, J. W. The reaction of the intervertebral disc to experimental lesions, 74
Warner, Francis James. The myelinization of the central nervous system of *Natrix sipedon*, 403
Warwick, R. Growth changes in the mental nerve and their relationship to the direction of the mental foramen, 72
— A study of retrograde degeneration in the oculomotor nucleus of the rhesus monkey, with a note on a method of recording it, 402
Weddell, G. The innervation of vibrissa-like hairs in the rabbit's leg, 69
— *See* Zander, E., joint authors, 78
Weddell, G. and Zander, E. Observations on the innervation of the cornea, 79
Whillis, J. *See* Bowden, R., joint authors, 76
Wilde, F. R. The anatomy of popliteal thrombosis, 74
Wilkinson, J. L. An oblique proximal septum of the pulp space, 409
Wolff, E. The distribution of the lacrimal fluid, 60
Wyburn, G. M. *See* Hutchison, J., joint authors, 67

Young, J. Z. *See* Fernand, V. S. V., joint authors, 402

Zander, E. and Weddell, G. Neurological techniques in relation to the anatomy of living corneal nerves, 78
— *See* Weddell, G., joint authors, 79
Zuckerman, S. and Ashton, E. H. The influence of geographical isolation on the teeth of the green monkey, 401
— *See* Breward, Madge M., joint authors, 68

VOLUME 84

CONTENTS OF PART 4

	PAGE
D. A. McDONALD AND J. M. POTTER. A method for the study of arterial anastomoses	327
E. L. PATTERSON. Sources of arterial blood supply to the superior and middle cervical sympathetic ganglia and the ganglion intermédiaire (with 9 Text-figures)	329
A. D. VIZOSO. The relationship between internodal length and growth in human nerves (with 11 Text-figures)	342
JAMES M. SPRAGUE AND MARGARET MEYER. An experimental study of the fornix in the rabbit (with Plates 1 and 2 and 7 Text-figures)	354
L. C. U. JUNQUEIRA. Alkaline and acid phosphatase distribution in normal and regenerating tadpole tails (with Plates 1 and 2)	369
N. A. BARNICOT. The local action of vitamin A on bone (with Plates 1 and 2 and 9 Text-figures)	374
MARGARET H. HARDY. The development *in vitro* of the mammary glands of the mouse (with Plates 1 and 2)	388
F. K. SANDERS. Anatomical note: Invasion of nerve homografts by regenerating muscle fibres (with 1 Text-figure)	394
IN MEMORIAM:	
SAMUEL ERNEST WHITNALL	395
ARTHUR BERRY APPLETON	397
Reviews	399
Proceedings, 1949–50	400
Index	411
Supplementary Index of Proceedings	414

INSTRUCTIONS TO CONTRIBUTORS

1. Manuscripts should be sent to the Editor of the *Journal of Anatomy*, Department of Anatomy, University College, Newport Road, Cardiff.

2. Manuscripts should be typewritten or written in very legible hand and must be fully corrected. Contributors will be responsible for the payment of any sum charged for the correction of the printer's proof in excess of 10s. per sheet (16 pages).

3. Illustrations must be in a finished form ready for reproduction. They must be properly lettered or labelled in type or by hand with reference pointers if necessary.

4. Line drawings must be sent whenever possible. Illustrations requiring half-tone blocks are very costly, *particularly under the present circumstances*.

5. The legend describing an illustration must be inserted in its appropriate place in the text and should not be placed on or appended to the drawing.

6. A contribution must terminate with a brief summary of the chief descriptions or observations and any conclusions drawn therefrom. The author is requested to provide the Editor with a typewritten abstract of his contribution not exceeding 3% of the text for publication in *Biological Abstracts*.

7. References are to be arranged in alphabetical order of the authors' names at the end of the contribution, the date of the paper being placed in brackets immediately after the author's name. Abbreviations for titles of journals given in the *World List of Scientific Periodicals* should be used. Full subject titles and first and last pages should be given. In the text a reference to a publication should be cited by giving the author, and, in brackets, the date, thus: "Allis (1934) found, etc."

8. The Editorial Committee cannot accept responsibility for damage to or loss of papers submitted to them. Contributors are advised to keep an additional copy of the manuscript and photographic reproductions of the illustrations.

9. Fifty gratis copies are given to each contributor.

10. The Journal is issued at the price of £2. 0s. 0d. per volume. Subscriptions, payable in advance, should be sent to the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1. Enquiries from the U.S.A. should be addressed to the Cambridge University Press American Branch, 51 Madison Avenue, New York, 10.